PB-226 955

REMOVAL OF NITRATE BY AN ALGAL SYSTEM, PHASE II

William R. Gianelli

California State Department of Water Resources
Sacramento, California

June 1971

DISTRIBUTED BY:

NTIS
National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

0052 04

WATER POLLUTION CONTROL RESEARCH SERIES • 13030 ELY 06/71-13

LIBRARY
Robert S. Kerr Water Research Laboratory
RECEIVED
MAR 2 [illegible]

PB 226 955

BIO-ENGINEERING ASPECTS OF AGRICULTURAL DRAINAGE

SAN JOAQUIN VALLEY, CALIFORNIA

REMOVAL OF NITRATE BY AN ALGAL SYSTEM

PHASE II

JUNE 1971

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

ENVIRONMENTAL PROTECTION AGENCY • RESEARCH AND MONITORING

CALIFORNIA DEPARTMENT OF WATER RESOURCES

139

BIO-ENGINEERING ASPECTS OF AGRICULTURAL DRAINAGE
SAN JOAQUIN VALLEY, CALIFORNIA

The Bio-Engineering Aspects of Agricultural Drainage reports describe the results of a unique interagency study of the occurrence of nitrogen and nitrogen removal treatment of subsurface agricultural wastewaters of the San Joaquin Valley, California.

The three principal agencies involved in the study are the Environmental Protection Agency, the United States Bureau of Reclamation, and the California Department of Water Resources.

Inquiries pertaining to the Bio-Engineering Aspects of Agricultural Drainage reports should be directed to the author agency, but may be directed to any one of the three principal agencies.

THE REPORTS

The first, three-year phase of the interagency study is to be reported upon in a series of twelve reports.

The second, one-year phase of the interagency study was limited to continued work on the two principal treatment methods. The second phase work develops design criteria and operational parameters for full-scale treatment facilities.

This report, "REMOVAL OF NITRATE BY AN ALGAL SYSTEM -- PHASE II", and the companion report, "DENITRIFICATION BY ANAEROBIC FILTERS AND PONDS -- PHASE II", contain the results of the second phase of the interagency study. These two reports are numbered sequentially, after the first twelve, in the series entitled "Bio-Engineering Aspects of Agricultural Drainage, San Joaquin Valley, California".

BIO-ENGINEERING ASPECTS OF AGRICULTURAL DRAINAGE
SAN JOAQUIN VALLEY, CALIFORNIA

REMOVAL OF NITRATE
BY AN
ALGAL SYSTEM
PHASE II

Prepared by

California Department of Water Resources
William R. Gianelli, Director

The agricultural drainage study was conducted under the direction of:

Robert J. Pafford, Jr., Regional Director, Region 2
UNITED STATES BUREAU OF RECLAMATION
2800 Cottage Way, Sacramento, California 95825

Paul DeFalco, Jr., Regional Director, Pacific Southwest Region
WATER QUALITY OFFICE, ENVIRONMENTAL PROTECTION AGENCY
760 Market Street, San Francisco, California 94102

John R. Teerink, Deputy Director
CALIFORNIA DEPARTMENT OF WATER RESOURCES
1416 Ninth Street, Sacramento, California 95814

DWR-WQO Grant 13030ELY6/71-13
DWR-USBR Contract #14-06-200-3389A
June 1971

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402
Price $2.10 domestic postpaid or $1.75 GPO Bookstore

REVIEW NOTICE

This report has been reviewed by the Environmental Protection Agency and the U. S. Bureau of Reclamation, and has been approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency or the U. S. Bureau of Reclamation.

The mention of trade names or commercial products does not constitute endorsement or recommendation for use by either of the two federal agencies or the California Department of Water Resources.

ABSTRACT

Major findings are presented from a one-year operational investigation conducted at the Interagency Agricultural Wastewater Treatment Center (IAWTC) on the use of algae to remove nitrogen from subsurface agricultural tile drainage in the San Joaquin Valley of California. The objectives of the study were to: (1) refine the design criteria, determined in a preliminary investigation, (2) develop operational procedures, and (3) recommend a design for a prototype algal nitrogen removal process.

The investigation demonstrated that the governing factors affecting the algal nitrogen removal process are the total amount of light available to the actively photosynthesizing algae and the influent nitrogen loading. Accordingly, if these two factors are known, the area required for maximum nitrogen removal can be approximated.

Turbid conditions, resulting from the suspension of non-photosynthesizing material during continuous or intermittent mixing, were found to be detrimental to the prolonged operation of the system. Maximal nitrogen assimilation also depended upon providing a completely balanced nutrient system, and varying amounts of supplemental carbon, phosphorus, and iron were required throughout the year.

Algal harvesting studies indicated that 90 percent or more of the algae could be removed throughout the year, under continuous operation, using a chemical-flocculent-sedimentation process but that the chemical additions required were dependent upon a number of algal growth factors.

Continuous operation of algal test units during 1970 showed the algal nitrogen removal process was capable of effectively reducing the influent nitrate-nitrogen concentration and other nutrients. The process reduced a varying influent nitrogen concentration of from 15 to 30 mg/l NO_3-N to 1 to 4 mg/l soluble effluent nitrogen throughout the year using varying operating parameters.

Recommendations are also given for the design and testing of "prototype" algal nitrogen removal plants using a modification of the stirred reactor design, and a proposed "slug-flow" algal nitrogen removal system designed to correct many of the inadequacies inherent in the first system.

BACKGROUND

This report is one of a series which presents the findings of intensive interagency investigations of practical means to control the nitrate concentration in subsurface agricultural wastewater prior to its discharge into other water. The primary participants in the program are the Water Quality Office of the Environmental Protection Agency, the United States Bureau of Reclamation, and the California Department of Water Resources, but several other agencies also are cooperating in the program. These three agencies initiated the program because they are responsible for providing a system for disposing of subsurface agricultural wastewater from the San Joaquin Valley of California and protecting water quality in California's water bodies. Other agencies cooperated in the program by providing particular knowledge pertaining to specific parts of the overall task.

The need to ultimately provide subsurface drainage for large areas of agricultural land in the western and southern San Joaquin Valley has been recognized for some time. In 1954, the Bureau of Reclamation included a drain in its feasibility report of the San Luis Unit. In 1957, the California Department of Water Resources initiated an investigation to assess the extent of salinity and high ground water problems and to develop plans for drainage and export facilities. The Burns-Porter Act, in 1960, authorized San Joaquin Valley drainage facilities as part of the State Water Facilities.

The authorizing legislation for the San Luis Unit of the Bureau of Reclamation's Central Valley Project, Public Law 86-488, passed in June 1960, included drainage facilities to serve project lands. This Act required that the Secretary of Interior either provide for constructing the San Luis Drain to the Delta or receive satisfactory assurance that the State of California would provide a master drain for the San Joaquin Valley that would adequately serve the San Luis Unit.

Investigations by the Bureau of Reclamation and the Department of Water Resources revealed that serious drainage problems already exist and that areas requiring subsurface drainage would probably exceed 1,000,000 acres by the year 2020. Disposal of the drainage into the Sacramento-San Joaquin Delta near Antioch, California, was found to be the least costly alternative plan.

Preliminary data indicated the drainage water would be relatively high in nitrogen. The then Federal Water Quality Administration conducted a study to determine the effect of discharging such drainage water on the quality of water in the San Francisco Bay and Delta. Upon completion of this study in 1967, the Administration's report concluded that the nitrogen

Preceding page blank

content of untreated drainage waters could have significant adverse effects upon the fish and recreation values of the receiving waters. The report recommended a three-year research program to establish the economic feasibility of nitrate-nitrogen removal.

As a consequence, the three agencies formed the Interagency Agricultural Wastewater Study Group and developed a three-year cooperative research program which assigned specific areas of responsibility to each of the agencies. The scope of the investigation included an inventory of nitrogen conditions in the potential drainage areas, possible control of nitrates at the source, prediction of drainage quality, changes in nitrogen in transit, and methods of nitrogen removal from drain waters including biological-chemical processes and desalination.

CONTENTS

Page

CONCLUSIONS 1
RECOMMENDATIONS 3

Chapter

I THEORY AND RATIONALE 5
  Introduction 5
  Phase I Conclusions 5
  Phase II Objectives 6
  Approach Rationale 7
  Theory of Algal Nitrogen Assimilation . . . 8
  Nitrogen Assimilation Mechanism 10
  Types of Algal Growth 10

II METHODS AND MATERIALS 13
  Experimental Procedures 13
  Analytical Methods 16
  Test Units 19

III RESULTS AND DISCUSSION 23
  Operational Procedures (Phase II, 1970) . . . 23
  Plant Influent 24
  Factors Affecting Nitrogen Assimilation . . . 26
  Algal Species and Predation 78
  Cellular Nitrogen 80
  Effluent Soluble Organic Nitrogen 83
  Operational Problems 84
  Pesticide Analysis 84
  Evaporation During 1970 86
  Diurnal Studies 86
  Operation Criteria -- 1970 88

IV ALGAL HARVESTING AND DISPOSAL 95
  Algal Harvesting 95
  Algal Disposal 105

V PROCESS EVALUATION 109
  Summary of the Phase II Investigation . . . 109
  Cost of Treatment 113

VI PROPOSED ALTERNATIVE TO THE MIXED-REACTOR SYSTEM STUDIED IN PHASES I AND II 115

ACKNOWLEDGEMENTS 121
REFERENCES 123
PUBLICATIONS 131

FIGURES

No. Page

1 Probable Nitrogen Pathways in Algal Growth Units 9
2 The Characteristic Pattern of Growth Shown by a Unicellular Alga in a Culture of Limited Volume 11
3 Projected Flow and Nitrogen Concentration of San Joaquin Valley Agricultural Wastewaters and Actual IAWTC Influent Nitrogen Concentration 13
4 Schematic of IAWTC Facilities 15
5 Volatile Solids vs Absorbance 18
6 Algal Growth Units at IAWTC 20
7 Monthly Variation in Total Dissolved Solids and Total Alkalinity in Plant Influent for 1970 25
8 Seasonal Variation in Total Dissolved Solids and Nitrate Nitrogen in Treatment Plant Influent 25
9 Effluent Nitrogen Concentration as Affected by Depth at Mean Detention Time 29
10 Effect of Carbon Dioxide Addition on Effluent Nitrogen Concentration at Three Depths 29
11 Quantitative Nitrogen Assimilation as Affected by Depth -- Grams per Day per Unit 30
12 Light Energy Received During 1970 31
13 Nitrogen Removal -- in Miniponds at Different Depths and Light Energy Levels 32
14 Nitrogen Assimilation in One-quarter-acre Unit at 24-inch Depth 33
15 Remaining Light Due to Wall Shading 34
16 Nitrogen Assimilation as Affected by Temperature 36

FIGURES (Continued)

No. Page

17 Volatile Solids Increase with Time as Affected by Temperature 37

18 Changes in Nitrate, Algal Cell Counts and Volatile Solids in Cultures of Different Volumes 38

19 Effect of Temperature on Algal Nitrogen Assimilation 39

20 Effect of Day or Night Mixing on Nitrogen Assimilation 40

21 Effect of Mixing and No Mixing With and Without Addition of Carbon Dioxide on Nitrogen Assimilation 41

22 Suspension of Solids During Mixing 42

23 Effect of Detention on Nitrogen Assimilation in 8-inch and 12-inch Units 43

24 Nitrogen Removed at Different Detention Times at 12-inch Depth with CO_2 44

25 The Effect of Various Levels of Aeration on Nitrate Removal in the Light Box 48

26 Effect of Surface/Volume Relationship and Various Types of Aeration on Cell Numbers at Six Days, Average of Five Replications . . 48

27 Typical Shift in pH and HCO_3^- in Algal Test Units 49

28 Changes in Total Influent Nitrogen and Effluent Total Soluble Nitrogen, Nitrite and Soluble Organic Nitrogen -- Average of All Units 50

29 Changes in Nitrite Concentration in Test Units With and Without CO_2 Addition 51

30 Changes in Total Influent and Effluent Nitrogen in Intermediate Detention Time Units With and Without Carbon Dioxide Addition 52

31 Carbon Dioxide Addition by Automatic pH Control 53

FIGURES (Continued)

No.		Page
32	Phosphate Solubility as a Function of pH	55
33	Solubility of Ferric Hydroxide in Water at 25°C	57
34	Solubility of Ferric Phosphate in Water at 25°C	57
35	Effect of Varying Iron and Phosphorus Concentrations on Nitrogen Assimilation -- Light Box Study	59
36	Effect of Iron or Phosphorus Addition on Nitrogen Assimilation with Carbon Dioxide Addition	61
37	Effect of Iron Addition on Nitrogen Assimilation Without Carbon Dioxide Addition	61
38	Influent and Effluent Phosphorus Concentrations in Units with Normal and Phosphorus-Supplemented Tile Drainage	62
39	Typical Sludge Accumulation in Test Units . . .	62
40	Iron and Phosphorus Solubility at Different pH Values	64
41	Preferential Algal Uptake of Ammonia Over Nitrate	67
42	Nitrogen Assimilation in Tile Drainage from Three Sumps	70
43	Comparison of Trace Metal Analysis of Filtered and Unfiltered Tile Drainage	73
44	Effluent Nitrogen Level in Soil Units	74
45	Effect of Biomass Control on Nitrogen Assimilation	77
46	Effect of a Nitrogen-deficient Medium on Algal Nitrate Assimilation	82
47	Evaporation minus Precipitation -- IAWTC, 1970	87

FIGURES (Continued)

No.		Page
48	Eight- and Twelve-inch Test Units with Lowest Effluent Nitrogen Concentration During 1970 . .	90
49	Average Soluble Nitrogen Removed During 1970 as Related to Detention Time and Depth	92
50	Average Soluble Nitrogen Removal at Various Depths in Intermediate Detention Time Units . .	93
51	Projected Acres Required at Different Nitrogen Concentration Necessary to Achieve a 2 - 4 mg/l Effluent Concentration -- April Operating Conditions and a Flow of 300 cfs	94
52	Projected Acres Required at Different Nitrogen Concentration Necessary to Achieve a 2 - 4 mg/l Effluent Concentration -- August Operating Conditions and a Flow of 300 cfs	94
53	Chemical Cost Required for Algal Concentration . .	98
54	Chemical Cost to Remove Total Suspended Solids to 40 mg/l During 1970	100
55	Effect of Bicarbonate Alkalinity on Iron Sulfate Required to Reduce Suspended Solids to 40 mg/l	101
56	Cost of Separation in Shallow Depth Sedimentation Unit During 1970	101
57	Schematic of Flotation Chamber Tested at IAWTC . .	102
58	Theoretical Nutrient and Light Availability to Algae in Two Types of Algal Reactors	116
59	Theoretical Algal Growth Phases in Two Types of Algal Reactors	116
60	Schematic Plan of Proposed Slug-Flow System . . .	118

TABLES

No. Page

1 Operational Schedule for Phase II Studies, 1970 14

2 Chemical Analysis Schedule 16

3 Percent of Total Pond Materials Found in Pond Sludge -- During Mixing 63

4 Average Dissolved Effluent Phosphate in Minipond During 1970 65

5 Summary of Micronutrient Trace Mineral Requirements 68

6 Trace Analysis -- 1970 72

7a Nitrate in the Water of Opaque and Clear Cylinders (Summer Test) 75

7b Nitrate in the Water of Opaque and Clear Cylinders (Fall Test) 76

8 Predominant Algal Species During 1970 at IAWTC . . 79

9 Average Percent Algal Cellular Nitrogen Found Under Different Growth Conditions in 1970 . . . 81

10 Average Effluent Concentration of Organic Nitrogen in Various Test Units During 1970 . . 83

11 Pesticide Analysis, 1970 85

12 Effect of Nutrient Addition and Mixing on Nitrogen Removal, 1970 Studies 89

13 Average Total Soluble Nitrogen Removed, 1970 . . 91

14 Estimated Average Chemical Cost per Million Gallons Separated (1970) (Jar Test Data) . . . 99

15 Estimated Algal Production by an Algal Stripping Plant, 1975-2000 106

16 Amino Acid Analysis 107

CONCLUSIONS

Based on the results of this investigation, the following may be concluded:

1. Removal of nitrogen from agricultural tile drainage using the algal nitrogen removal process is a technically feasible process on a year-round basis.

2. The total amount of soluble nitrogen in the plant effluent will probably vary between 2 and 4 mg/l, depending on the influent nitrogen loading and environmental conditions. In addition, from 1 to 2 mg/l algal cellular nitrogen can be expected in the plant effluent, depending upon the degree of removal of suspended solids in the separation process.

3. The principal factors affecting maximum algal nitrogen assimilation are the total amount of light available to the actively photosynthesizing algae and the influent nitrogen loading.

4. Depending upon the total available light and nitrogen loading, detention times required for maximum nitrogen removal during 1970 varied between 5 and 16 days, and operating depths varied between 8 and 12 inches.

5. Turbid conditions resulting from the suspension of nonphotosynthesizing material during continuous or intermittent mixing of an algal reactor are detrimental to the prolonged operation of the system.

6. The optimal carbon-to-nitrogen nutrient ratio in the system is approximately 5 to 1. If the carbon demand exceeds the carbon available in the growth medium, supplemental carbon can be added to the system as carbon dioxide, or possibly as bicarbonate. In applying supplemental carbon, carbon dioxide should be added to the system only during the two- or three-hour period of peak photosynthesis. Possibly bicarbonate may be effectively applied by adding it continuously to the influent.

7. It appears from the data that a carbon deficiency in the algal system partially blocks the cellular reduction of nitrate to ammonia resulting in the excretion of nitrite into the growth medium.

8. Phosphate is almost completely removed from the system by algal phosphate assimilation and/or chemical precipitation resulting from photosynthetically induced increases in pH.

9. Iron is required in the algal system and affects both nitrogen uptake and algal suspension.

10. More than 90 percent of the algae can be separated on a year-round basis by the chemical-flocculation-sedimentation process.

11. Algae can be differentially separated to provide a product of variable inorganic and organic composition.

RECOMMENDATIONS

1. At least two one-half to one million gallon per day parallel "prototype" algal nitrogen removal plants should be constructed and operated to permit investigation of "plug flow" versus completely mixed systems; lined versus unlined systems; and systems with biomass control versus no biomass control. Such systems would be provided with equipment for carbon, iron and phosphate addition in line as needed, and for pH control to facilitate the availability of iron and phosphorus to the algae.

The prototype facility should be equipped for algal harvesting so that the integration of growth and harvesting may be further investigated by the promising harvesting methods found in the recent investigation.

Operation of the prototype plants should be conducted for at least two years to determine variations in climatic conditions and waste compositions that may normally occur.

If possible, the prototype plants should be located so that they could be used either for extended pilot investigations to continuously update the technology of nutrient removal or as integral parts of the algal nitrogen removal system.

2. Because even small changes in permissible nitrogen discharge levels may profoundly affect process costs, nitrogen discharge requirements in the western Delta-Suisun Bay area should be studied further to determine if seasonal or other changes in discharge requirements could be made.

CHAPTER I

THEORY AND RATIONALE

Introduction

This report presents the major findings of a one-year operational investigation conducted at the Interagency Agricultural Wastewater Treatment Center (IAWTC) at Firebaugh, California, by the United States Bureau of Reclamation (USBR), the Environmental Protection Agency (EPA), and the California Department of Water Resources (DWR) on the use of algae to remove nitrogen from agricultural tile drainage in the San Joaquin Valley of California. This investigation, which represents only one aspect of an overall program (1, 2, 3, 4), was established to determine if a method(s) could be found to reduce the total nitrogen content in agricultural tile drainage (5, 6, 7, 8) to 2 mg/l or less, the level established as a maximum for the San Francisco Bay-Delta area (9, 10).

Phase I Conclusions

The algal nitrogen removal investigation was conducted in two phases. Phase I (11) was designed to determine the feasibility of using controlled algal growth and harvesting to remove nitrate-nitrogen from agricultural tile drainage (12, 13, 14, 15, 16, 17). That study concluded:

1. Algal growth and harvesting is a technically feasible method of removing nitrate-nitrogen from subsurface tile drainage.

2. Preliminary costs of an algal system, as studied at the IAWTC, would be approximately $135 per million gallons of water treated.

3. Some nutrients (e.g., carbon, phosphorus, and iron) may be limiting in tile drainage during portions of the year and would have to be supplemented in an algal treatment plant to achieve maximum nitrogen assimilation and removal.

4. No more than four hours of mixing (with velocities of from 0.25 to 0.50 feet per second) would be required for maximum nitrogen assimilation.

5. Theoretical detention time required for maximum nitrogen assimilation would be from 5 to 16 days and appeared to be inversely related to pond temperatures within the range of 12°C to 25°C, and independent of temperature within the range of 25°C to 33°C.

Preceding page blank

6. Optimum culture depth was 8 inches, but this depth could be increased by lengthening the detention time. Comparison of different depths (8 to 16 inches) showed that nitrogen assimilation varied seasonally and was directly related to available light.

7. A secondary study at the IAWTC indicated a symbiotic process (algal-bacterial) removed substantial amounts of nitrogen and should be considered as a potential nitrogen removal process.

8. Under the study conditions, some mechanism to control sludge accumulation appeared to be required during certain times of the year.

9. Algae can be separated readily from agricultural tile drainage by lower levels of flocculents (e.g., ferric sulfate, 5 mg/l; alum, 20 mg/l; lime, 40 mg/l; and cationic polyelectrolytes, 0.2 mg/l) than found necessary for harvesting sewage-grown algae (18). This concentrated algae slurry can then be dewatered and dried to produce a stable product.

10. Removal of nitrogen (by any method) from agricultural tile drainage does reduce the stimulatory effect of drainage waters on algal growth in the receiving waters (San Francisco Bay-Delta).

11. If a market were developed for the algal product, the product would probably be worth approximately $80 to $100 per ton, an amount that could be subtracted from the overall cost of treatment.

12. Phase II studies should be initiated to define operational criteria.

Phase II Objectives

At the completion of the Phase I studies in December 1969, the Interagency Advisory Group authorized initiation of the Phase II (operational) studies to be conducted at the IAWTC from January 1970 through December 1970. The primary objectives of the Phase II studies were to:

1. Refine the design criteria.
2. Develop operational procedures.
3. Recommend a design for a prototype algal nitrogen removal plant.

Although this report, Phase II, deals specifically with the 1970 operational studies at the IAWTC, Phase I results have been included where applicable.

Approach Rationale

The Phase I studies were designed to determine the feasibility of using algae for the removal of nitrogen from agricultural tile drainage. After preliminary studies were conducted to define the basic operational factors that might be expected to affect the efficiency of nitrogen assimilation by the algae, a series of short-term investigations was conducted to determine the effect of: (1) mixing, (2) detention time, (3) depth, and (4) nutrient addition (carbon, phosphorus, and iron) on the operation of algal growth units. From the results of these studies, which are presented in the introduction of this report, it was concluded that algal nitrogen removal is a feasible method of removing nitrogen from agricultural tile drainage.

The Phase II studies were designed to follow the predicted month-by-month nitrogen loading for San Joaquin Valley agricultural wastewaters during one year's operation. The goal was to develop the basic techniques needed to determine the seasonal operating criteria required in a full-scale plant, rather than to maximize nitrogen assimilation in each unit. Seasonal requirements were determined by monitoring algal nitrogen assimilation over an entire year in growth units operating on various combinations of growth regulating parameters (see Table 1). In working toward these goals, the following questions had to be answered before an assessment of the process could be obtained.

1. Could the operating criteria governing nitrogen assimilation determined in the Phase I studies be applied effectively to the Phase II studies?

2. Would any of the basic operating criteria determined in Phase I change over an extended period of time?

3. Could sustained nitrate-nitrogen removal be obtained on a long-term basis?

4. What levels of nitrogen assimilation could be expected during different times of the year?

5. What are the optimal operational variables required for maximum nitrogen removal throughout the year?

6. Would the algal harvesting criteria change?

7. What type of algal plant design would be optimal for maximum nitrogen removal?

The present investigation was designed to answer these and other such questions pertaining to the continuous operation of an algal nitrogen removal system.

Theory of Algal Nitrogen Assimilation

Although algae have been used for a number of years in the secondary treatment of domestic sewage in oxidation ponds, only recently has their application to tertiary treatment (nutrient removal) of wastewaters been considered. Shelef (19) has stated that the major advantage of using algae in wastewater treatment is the simultaneous accomplishment of biomass production (by-product), oxygen production, carbon dioxide adsorption, and nutrient assimilation. Furthermore, algae are utilized in preference to higher plants (both would achieve the same overall results) because they have a high specific growth rate and continuous reproduction, and can be grown in a continuous culture on a year-round basis.

Although there are numerous ways to represent the overall stoichiometrics of algae photosynthesis and nutrient assimilation, Equation [1] proposed by Jewell and McCarty (20) best seems to represent the algal nitrogen removal process investigated at the IAWTC:

$$aCO_2 + cNO_3 + ePO_4^{-3} + (c+3e)H^+ + 1/2\,(b-c-3e)H_2O + \text{sunlight} = C_aH_bN_cO_dP_e + (a + b/4 + 5c/4 - d/2 + 5e/4)O_2 \qquad \text{Eq. [1]}$$

This equation predicts that as plant photosynthesis occurs, a fixed ratio of major nutrients will be eliminated from the growth media. For example, the following equation by McCarty (21) represents the average stoichiometric incorporation of C, H, O, N, and P into plant cell material:

$$106\ CO_2 + 81\ H_2O + 16\ NO_3^- + HPO_4^= + 18\ H^+ + \text{light} = C_{106}H_{181}O_{45}N_{16}P + 150\ O_2 \qquad \text{Eq. [2]}$$

Equation [2] represents the algal composition typically reported in the literature (22, 23, 24, 25, 26, 27) for algae under good growing conditions and is usually about 6 to 11 percent nitrogen, 50 percent carbon, and 0.5 to 2 percent phosphorus. However, the chemical composition of algae can be extremely variable, differing with species and conditions of growth (19, 26, 28, 29). For example, Foree and McCarty (30) found that the cellular composition reported for Chlorella pyrenoidosa varied from $C_{6.20}H_{10.2}O_{3.32}N$ to $C_{57.3}H_{103.2}O_{10.0}N$.

The extent of algal cellular production, Equation [1], is proportional to the concentration of the limiting nutrient (22) and corresponds to "Lieberg's Law of the Minimum" and Blackman's concept of "Limiting Factors". The rate at which this reaction will proceed is a function of available light energy, which is the determining factor regulating algal plant capacity, physical dimensions, operational parameters, biomass concentration and production (19, 31, 32). Accordingly, the ultimate goal of design in an algal nutrient removal system should be the optimal utilization of light with only the undesirable nutrient as the limiting factor.

The probable nitrogen removal pathways in the algal growth units as studied at the IAWTC are presented in Figure 1.

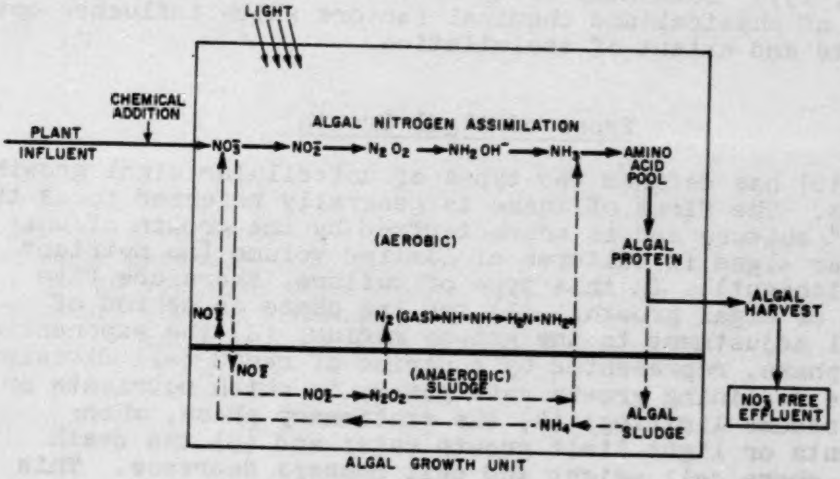
LIGHT
CHEMICAL ADDITION
PLANT INFLUENT
ALGAL NITROGEN ASSIMILATION
NO_3^-
NO_2^-
N_2O_2
NH_2OH^-
NH_3
AMINO ACID POOL
(AEROBIC)
ALGAL PROTEIN
ALGAL HARVEST
NO_2^-
N_2 (GAS) → NH=NH → H_2N−NH_2
NO_2^-
(ANAEROBIC) SLUDGE
NO_2^-
N_2O_2
NH_4
ALGAL SLUDGE
NO_3^- FREE EFFLUENT
ALGAL GROWTH UNIT

FIGURE 1 - PROBABLE NITROGEN PATHWAYS IN ALGAL GROWTH UNITS

If such a system is hydraulically balanced, the only pathway utilized will be the algal uptake and assimilation of nitrate-nitrogen, with subsequent conversion to cellular material and the removal of the algae (and incorporated nutrients) from the growth unit. This may not be the case in large-scale units. Possible secondary nitrogen removal pathways are presented in Figure 1 and their possible significance will be presented in Chapter III of this report.

Nitrogen Assimilation Mechanism

In general, algae can utilize inorganic nitrogen as either ammonia, nitrate, or nitrite. The reduction process by which inorganic nitrogen is utilized requires energy in the form of light (Equation [1]) and is temperature dependent. The level of energy required depends upon the amount and type of nitrogen reduction required.

The removal pathways of nitrate-nitrogen (the predominant form in the tile drainage at the IAWTC) from the growth medium via conversion into cellular material and subsequent removal from the growth unit are illustrated in Figure 1. The incorporation of nitrate-nitrogen into the cell involves a chain of enzymatic reactions by which the nitrate ion is first pumped into the cell, reduced to ammonia via several reductase enzymes, and finally incorporated into chlorophyll, nucleic acids, amino acids, and proteins (19, 21, 23, 24, 28, 33, 34, 35). Each step of the process is affected by a number of physical and chemical factors which influence both the rate and extent of assimilation.

Types of Algal Growth

Fogg (36) has defined two types of unicellular algal growth systems. The first of these is generally referred to as the "batch" culture and is characterized by the growth of unicellular algae in cultures of limited volume (no nutrient replenishment). In this type of culture, there are five phases of algal growth: (1) the lag phase or period of initial adjustment to the growth medium; (2) the exponential (log) phase, represented by a period of rapid cell division; (3) the declining growth rate phase, in which nutrients or light become limiting; (4) the stationary phase, when nutrients or light limit growth rate; and (5) the death phase, where cell weight and cell numbers decrease. This constitutes the normal pattern of growth for cultures of limited volume; however, if the culture is not unialgal, the original species may be replaced in the later phases of growth by algal species with slightly different nutritional requirements. Figure 2 illustrates the batch-culture type of growth with unicellular algal cultures.

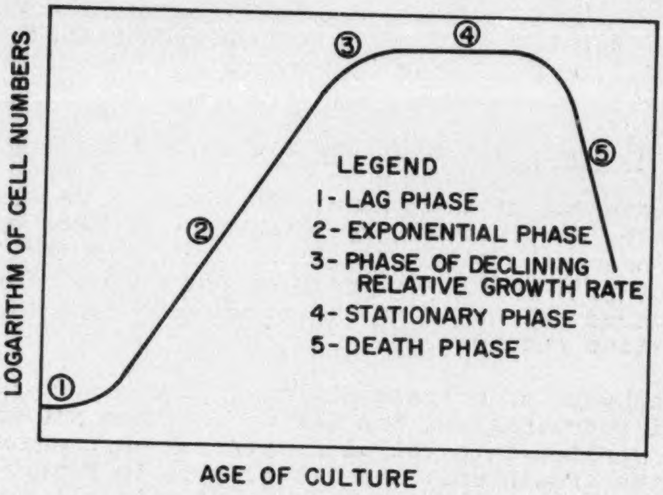

FIGURE 2 - THE CHARACTERISTIC PATTERN OF GROWTH SHOWN BY A UNICELLULAR ALGA IN A CULTURE OF LIMITED VOLUME

The specific growth rate of cells during the exponential phase is a function of cell concentration and can be described by the following Equation [3]:

$$\frac{dN}{dt} = KN \qquad \text{Eq. [3]}$$

Where K is the specific growth rate (day^{-1}), N is the cell concentration (in any applicable unit), and t is the time in days.

The second type of algal growth system encountered is usually referred to as "continuous flow" culture and is characterized by maintenance of the exponential phase (previously described) of algal growth by continual replenishment of the algal nutrients. In this system the algal population density is held at a relatively constant (steady-state) level by manipulation of the growth factors. This is the type of system most commonly used in experimental wastewater treatment plants utilizing algae and in investigations dealing with algal growth kinetics (19, 25, 37, 38, 39). As will be demonstrated in a later section, the growth units utilized in the IAWTC studies, though designed as continuous flow types, were in actuality somewhere between batch and continuous flow.

CHAPTER II

METHODS AND MATERIALS

The equipment and methods used in the IAWTC studies were described in detail in the Phase I report. A brief resume, as pertains to the Phase II studies, is as follows.

Experimental Procedures

The basic experimental test units utilized in the Phase II investigation along with the operating criteria of each unit during 1970 are listed in Table 1. Although several units were retained for special studies, the majority of experimental test units were held on fixed operating schedules for the entire year. The large 1/4-acre algal growth pond, described in Phase I, was retained as a flexible unit, serving as both a demonstration and algal production unit with which algal separation could be studied on a seasonal basis.

The predicted changes in nitrogen concentration for San Joaquin Valley agricultural wastewaters as well as the IAWTC plant influent nitrogen levels are shown in Figure 3. An

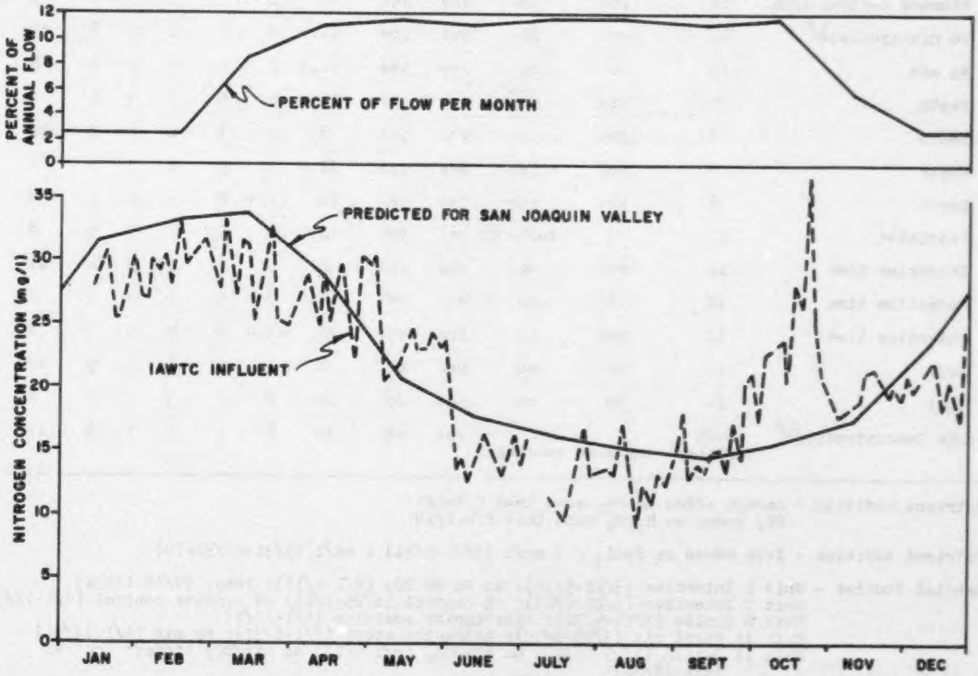

FIGURE 3-PROJECTED FLOW AND NITROGEN CONCENTRATION OF SAN JOAQUIN VALLEY AGRICULTURAL WASTEWATERS AND ACTUAL IAWTC INFLUENT NITROGEN CONCENTRATION

TABLE 1

OPERATIONAL SCHEDULE FOR PHASE II STUDIES
1970

Unit	Investigation	Depth (Inches)	Mixing 0800-0830 1200-1530	Nutrient Addition[1]			Detention Times (Days)							
				CO_2	PO_4	Fe[2]	3/25	5/1	5/18	7/1	9/1	9/18	10/19	11/17
1	Special[3]	12	yes	no/yes	yes	variable	11.4	8	5	3	3	3	8	10
2	Special[3]	12	yes	yes	yes	variable	11.4	8	5	3	3	5	8	10
3	No PO_4	12	yes	yes	no	yes	11.4	8	5	3	3	5	8	10
4	No Iron	12	yes	yes	yes	no	11.4	8	5	3	3	5	8	10
5	Detention time	12	yes	yes	yes	yes	8	5	3	1	2	8	12	15
6	Detention time	12	yes	yes	yes	yes	11.4	8	5	3	3	3	4	5
7	Detention time	12	yes	yes	yes	yes	16	11.4	8	5	5	5	8	10
8	Special[3]	12	-	yes/no	yes	yes	8	5	5	3	3	5	8	10
9	Biomass control	12	yes	yes	yes	no	8	5	5	3	3	5	8	10
10	Biomass control-iron	12	yes	yes	yes	yes	8	5	5	3	3	5	8	10
11	No mix-special[3]	12	no	no	yes	yes	11.4	8	5	5	5	8	12	15
12	No mix	12	no	no	yes	yes	11.4	8	5	3	3	5	8	10
13	Depth	16	yes	yes	yes	yes	11.4	8	5	3	3	5	8	10
14	Depth	8	yes	yes	yes	yes	8	5	3	1	2	8	12	15
15	Depth	8	yes	yes	yes	yes	11.4	8	5	3	3	3	4	5
16	Depth	8	yes	yes	yes	yes	16	11.4	8	5	5	5	8	10
17	Special[3]	12	-	variable	yes	yes	11.4	8	5	3	3	5	8	10
18	Detention time	12	yes	no	yes	yes	8	5	3	1	2	8	12	15
19	Detention time	12	yes	no	yes	yes	11.4	8	5	3	3	3	4	5
20	Detention time	12	yes	no	yes	yes	16	11.4	8	5	5	5	8	10
21	Soil	12	no	no	yes	no	8	5	3	1	3	5	12	15
22	Soil	12	no	no	yes	no	11.4	8	5	3	3	3	8	10
1/4-acre Demonstration[4]		8-24 variable	variable	variable	yes	yes	15	8	5	5	7	8	10	15

---

[1] Nutrient Addition - Carbon added as CO_2 such that C/N=5/1
PO_4 added as H_3PO_4 such that P/N=1/10

[2] Nutrient Addition - Iron added as $FeCl_3$ - 3 mg/1 (3/25-5/31) 1 mg/1 (5/31-12/31/70)

[3] Special Studies - Unit 1 Intensive-(3/21-5/31); No Fe or CO_2 (6/1-9/17); Temp. (9/18-12/31)
Unit 2 Intensive-(3/21-6/24); pH control (6/25-7/1); pH biomass control (7/1-12/31)
Unit 8 Cyclic (3/21-5/31); bicarbonate addition (6/1-12/1)
Unit 11 Night mix (3/25-6/30); hydraulic study (7/1-8/31); no mix (9/1-12/31)
Unit 17 8-inch (3/25-5/31); no mix/CO_2 (6/1-8/1); no mix/CO_2 biomass control (8/1-12/31)

[4] 1/4-acre demonstration unit: detention time 12-inch (3/25-7/31); 24-inch (8/4-11/16); 8-inch (11/16-12/31)

attempt was made to duplicate the predicted monthly changes in influent nitrogen, although loading could only be approximated (detention times from the Phase I study were used as a guide).

A schematic diagram of the IAWTC algal nitrogen removal facilities is presented in Figure 4. As shown in this figure, there were 22 experimental test units referred to as minipodns, each with a surface area of 128 square feet and a water volume of 1,000 gallons at a 12-inch depth, and a 1/4-acre demonstration unit. The plant influent came from a common storage pond and the carbon dioxide was supplied to all units through a common manifold system.

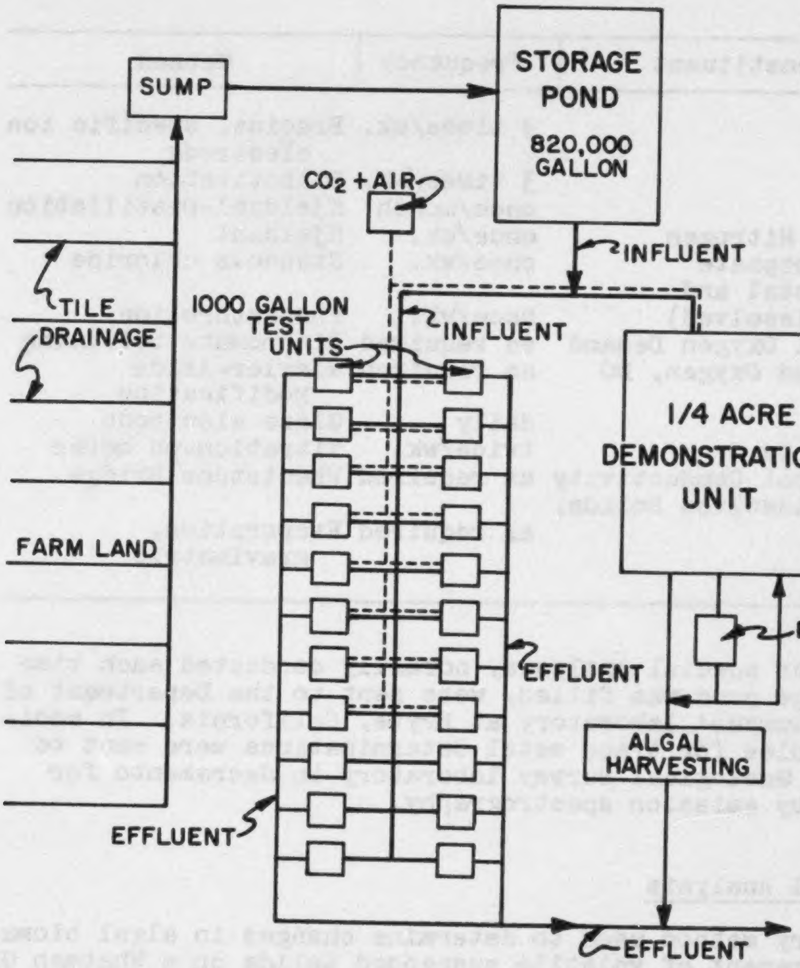
SUMP
STORAGE POND
820,000 GALLON
CO_2 + AIR
INFLUENT
TILE DRAINAGE
1000 GALLON TEST UNITS
INFLUENT
1/4 ACRE DEMONSTRATION UNIT
FARM LAND
BIOMASS REGULATION-SEPARATION UNIT
EFFLUENT
ALGAL HARVESTING
EFFLUENT
EFFLUENT

FIGURE 4 - SCHEMATIC OF IAWTC FACILITIES

Analytical Methods

Chemical Analysis

Chemical analyses (Table 2) of treated and untreated tile drainage were made according to the various procedures outlined in Standard Methods (40) for the determination of the chemical constituents considered pertinent to the investigation.

TABLE 2

CHEMICAL ANALYSIS SCHEDULE

Constituent	Frequency	Method
Nitrate	3 times/wk.	Brucine, specific ion electrode
Nitrite	3 times/wk.	Diazotization
Ammonia	once/month	Kjeldahl-Distillation
Organic Nitrogen	once/wk.	Kjeldahl
Orthophosphate	once/wk.	Stannous chloride
Iron (total and dissolved)	once/wk.	Phenanthroline
Chemical Oxygen Demand	as required	Dichromate refluxing
Dissolved Oxygen, DO	as required	Winkler-Azide Modification
pH	daily	Glass electrode
Alkalinity	twice/wk.	Titration-pH meter
Electrical Conductivity	as required	Wheatstone Bridge
Total Dissolved Solids, TDS	as required	Evaporation, gravimetric

Samples for special analyses, normally conducted each time the storage pond was filled, were sent to the Department of Water Resources' laboratory at Bryte, California. In addition, samples for trace metal determinations were sent to the U. S. Geological Survey laboratory in Sacramento for analysis by emission spectrography.

Biological Analysis

The primary method used to determine changes in algal biomass was measurement of volatile suspended solids on a Whatman GFA glass filter disc; in addition, all units were examined at least once a week to observe the condition and species of algae present. At the same time as the species examination, cell counts were determined by a microscope and a hemacytometer.

In some light box studies, the progress of cell growth was followed by measuring in vivo chlorophyll fluorescence. A Turner Model III fluorometer was modified by adding a blue light source and the proper combination of filters (Corning CS5-60 primary and CS2-60 secondary) for measurement of chlorophyll a.

Physical Analysis

A continuous recording analyzer was used to monitor the 1/4-acre demonstration unit for water temperature, pH, and sunlight. Each of these parameters was also measured routinely in the smaller experimental test units. In addition, a weather station was located on the site to record daily changes in air and water temperature, evaporation, precipitation, and wind.

Quality Control

Quality control was conducted routinely and analytical techniques were corrected, if not within the limits suggested in Standard Methods (40). Because many of the results used in the investigation were based on changes in nitrate-nitrogen as measured with the use of a specific ion electrode, special mention should be made of this method of nitrate-nitrogen analysis.

This instrument was standardized against known concentrations of nitrate in denitrified tile drainage as well as with nitrate standards. Usually, a plot of meter readings versus concentration showed a straight line between 0.5 and 50 mg/l nitrate-nitrogen. Although some problems were encountered as a result of changes in total dissolved solids (TDS), as well as some day-to-day variations in electrode response, the specific ion electrode was considered rapid (up to 150 analyses per hour), simple, and reliable.

Data Analysis

In the following sections, many of the results are reported as nitrogen assimilated, expressed either as mg/l or as percent removed. In general, "nitrogen assimilation" is expressed as the amount of soluble nitrogen disappearing from the medium as a result of assimilation and conversion to algal cellular material. However, this method of expressing nitrogen assimilation is a simplification of a very complex system where, for example, algal cellular production and decomposition occur simultaneously.

In certain instances, response of algal growth to nutrient addition has been expressed as changes in volatile solids (VS), *in vivo* fluorescence, absorbance, or changes in some constituent other than nitrogen. A typical example is that shown by the correlation of volatile solids to absorbance, Figure 5. Similar correlations of algal biomass changes to nitrogen assimilation have been determined for the other items.

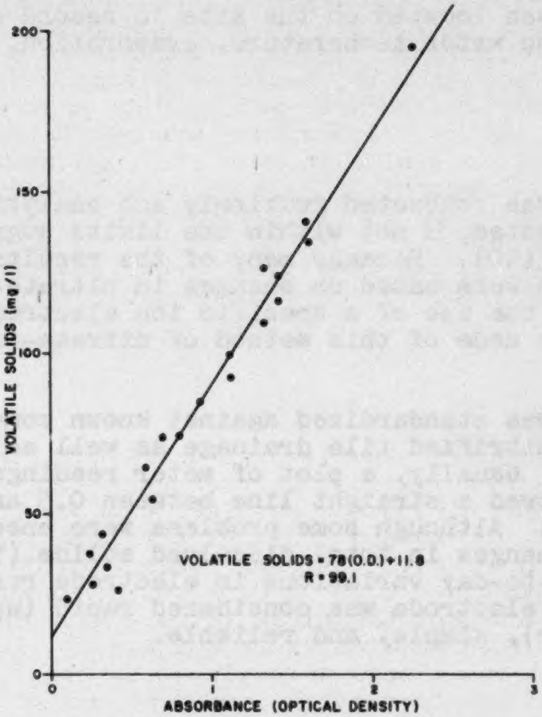

FIGURE 5- VOLATILE SOLIDS Vs ABSORBANCE

Test Units

Flask Bioassays

Algal nutrient bioassays, as well as special studies, were conducted routinely by batch culture techniques. These studies were customarily conducted with duplicate or triplicate 1,000 milliliter (ml) Erlenmeyer flasks containing 500 ml of the medium to be tested. Small concentrations of algae (2,000 to 3,000 cells/ml), usually *Scenedesmus quadricauda*, taken from the outdoor growth units were used as the inoculum. The inoculum was not axenic nor even unialgal, although it normally contained 90 to 95 percent *Scenedesmus*. The use of algae from the test units proved to be an effective method of monitoring growth variables seasonally, perhaps because the algae were acclimatized.

Lighting was usually continuous (300- to 400-foot candles at the medium surface), and temperature, unless specifically altered, was held at 22± 4°C. No means of automated mechanical agitation was provided, but air (compressed only or enriched with carbon dioxide) could be introduced to individual flasks via a central manifold to provide agitation and supplemental carbon. Air volume was regulated by short sections of capillary tubing in each line and resulted in approximately equal amounts of air being delivered to each flask (41).

Analyses similar to those previously described for the outdoor growth units (Table 2) were conducted during these studies.

Operational Units

Miniponds. The primary experimental units utilized in the Phases I and II investigations were 22 resin-coated plywood growth units. Each minipond was 8 feet wide by 16 feet long, with a surface of 128 square feet and a volume of 1,000 gallons at a 12-inch depth. Mixing pumps with 80-gallon-per-minute capacity provided 0.25- to 0.5-foot-per-second (fps) velocities to re-suspend the algae (Figure 6). Three pond depths were studied with these units -- 8, 12, and 16 inches. In addition, timers were placed in the electrical circuits of the pumps to vary the hours of mixing during a 24-hour period. (The standard mixing schedule in Phase II was from 8 to 8:30 a.m. -- sampling period -- and from 12 noon to 3:30 p.m. -- peak photosynthetic period.)

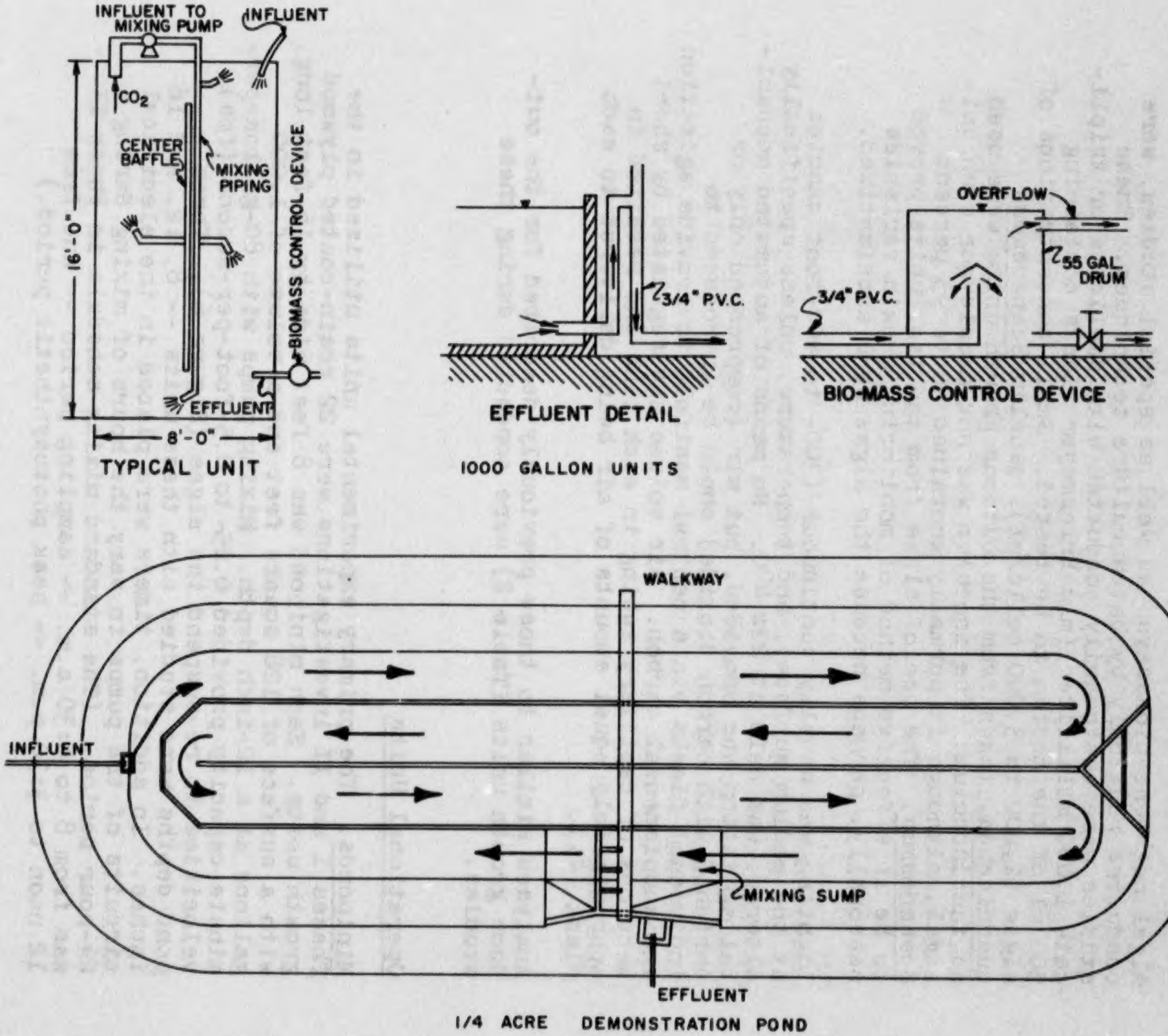
INFLUENT TO MIXING PUMP
INFLUENT
CO_2
CENTER BAFFLE
MIXING PIPING
BIOMASS CONTROL DEVICE
EFFLUENT
16'-0"
8'-0"
TYPICAL UNIT
3/4" P.V.C.
EFFLUENT DETAIL
1000 GALLON UNITS
OVERFLOW
55 GAL. DRUM
3/4" P.V.C.
BIO-MASS CONTROL DEVICE
WALKWAY
INFLUENT
MIXING SUMP
EFFLUENT
1/4 ACRE DEMONSTRATION POND

FIGURE 6 - ALGAL GROWTH UNITS AT IAWTC

Tile drainage was individually metered from a storage pond supply at rates to provide preselected detention times based on the Phase I studies. This plastic-lined storage pond had an 820,000-gallon capacity and was refilled from a tile drainage field adjacent to the IAWTC site.

Nitrate-nitrogen was mixed into the storage pond as required to build up the nitrogen level to the predicted concentration (Figure 3). No adjustment was made when the wastewater nitrogen level was in excess of predicted.

The effluent from the test units was drawn from near the bottom of each unit (opposite the influent) and discharged through a "broken" siphon arrangement (Figure 6). This effluent tube was also used to maintain a constant depth in the unit.

The ponds receiving supplemental carbon (as carbon dioxide) had a mixture of atmospheric air and varying levels of carbon dioxide metered into the intake side of the mixing pumps during the afternoon mixing cycle. The carbon concentration corresponded to the unit's nitrogen loading. To assure complete addition to the test units, the bicarbonate forms of carbon, as well as phosphorus and iron, were added to individual test units daily, rather than to the central storage pond supply.

An attempt was made to control algal biomass in several of the test units by converting a 55-gallon drum into a settling tank (Figure 6). The water from the growth unit was cycled through the drum where some of the suspended material settled out and then the supernatant was returned to the growth unit. The sludge from each of these separation units was periodically collected, measured, and chemically analyzed.

One-quarter Acre Demonstration Unit. The 1/4-acre unit was an asphalt-lined pond with a 12.5-foot-wide folded raceway channel approximately 800 feet long. The 4-foot center baffles were constructed of aluminum siding attached to a wooden upright frame. This unit could be operated at depths varying from 0.5 to 3 feet. The effluent could be taken from either the top or near the bottom of the mixing sump (Figure 6). With its four available mixing pumps, operating velocities of up to one foot per second were theoretically possible at all operating depths. As with the smaller test units, each pump had a timer which allowed for an almost infinite variety of mixing schedules. To provide supplementary carbon, an air-carbon dioxide mixture could be metered into the intake side of the mixing pumps.

CHAPTER III

RESULTS AND DISCUSSION

Operational Procedures (Phase II, 1970)

Operational Studies

The Phase II operational investigation was designed to determine the seasonal effect of: (1) phosphate, (2) carbon, (3) iron, (4) mixing, (5) biomass regulation, (6) depth, (7) detention time, (8) soil, (9) light, and (10) temperature on nitrogen assimilation by algae in tile drainage. The purpose of the investigation was not to operate each unit at maximum nitrogen removal efficiency but to determine which combination(s) of the above variables provided maximum nitrogen assimilation under different environmental conditions. Good experimental design dictated that only one combination of variables be optimal over a given unit time. Consequently, a decision was made early in the investigation to adhere to the preplanned study design, except for special units, regardless of the results in individual test units.

Flask Bioassays

During the IAWTC investigation (Phases I and II), a number of light box algal bioassay studies were conducted which were designed to determine factors that might alter the level or extent of nitrogen assimilation by algae in agricultural tile drainage. The results from these studies were then applied to the operation of the miniponds. In general, the algal bioassays proved to be a rapid and effective method of evaluating nitrogen assimilation under different growth conditions. Summaries of these studies, considered pertinent to an understanding of the algal process, are presented in the following sections.

1970 Startup

Both the miniponds and the 1/4-acre demonstration unit were continuous flow (influent injected in one end, effluent removed at the other end), stirred (semi-mixed) algal reactors.

At the termination of the Phase I studies in December 1969, all of the miniponds except the units containing a layer of soil were drained, cleaned, repaired, and refilled with tile drainage containing a Scenedesmus inoculum from the 1/4-acre

Preceding page blank

demonstration unit. This procedure was completed in February 1970. The units were then operated on batch for several weeks before placing them on the designated schedule. During the next month and a half, there was a transition period between Phase I and Phase II which resulted in a number of operational items being neglected. By mid-March a large error in the rate of influent to many of the units was detected. In addition, a volume measurement of the air-carbon dioxide to each test unit showed that the flows were in error. By the second week of April, these operational discrepancies had been largely corrected and this resulted in a corresponding improvement in nitrate assimilation.

From mid-April through December 1970, most of the miniponds were operated continuously on the designated schedule. Detention time was varied in an attempt to bracket seasonal changes in optimal detention time. The remaining units were retained for special studies. At the end of July, the majority of the units were emptied and restarted with inoculum from the 1/4-acre demonstration unit.

Plant Influent

Subsurface agricultural tile drainage (46) was pumped to an 820,000-gallon covered storage pond which provided the influent for the algal nitrogen removal studies. Although the main reason for having a storage pond was to assure a source of influent, it was also intended to provide a constant water quality to the test units. There were, however, significant changes in TDS and general water quality each time the pond was filled. The changes in TDS and total alkalinity in the plant influent for 1970 are plotted in Figure 7. The large change in TDS noted at the end of the summer resulted from the crop rotation and water application practices in the tile drainage field adjacent to the test site, which coincided with changes in major nutrients that had occurred over a three-year period at the IAWTC (Figure 8).

When the storage pond was filled with tile drainage, samples were collected and analyzed for standard minerals, trace elements (thought to be required for algal growth), pesticides, and algal bioassay nutrient responses to carbon, phosphorus, and iron addition.

Late in 1970, the aluminum and iron levels in the plant influent increased noticeably. A check of the storage pond roof (which was aluminum supported by iron girders) indicated that there was a considerable amount of corrosion which was probably responsible; however, it is not known whether this had any effect on algal growth and nitrogen metabolism.

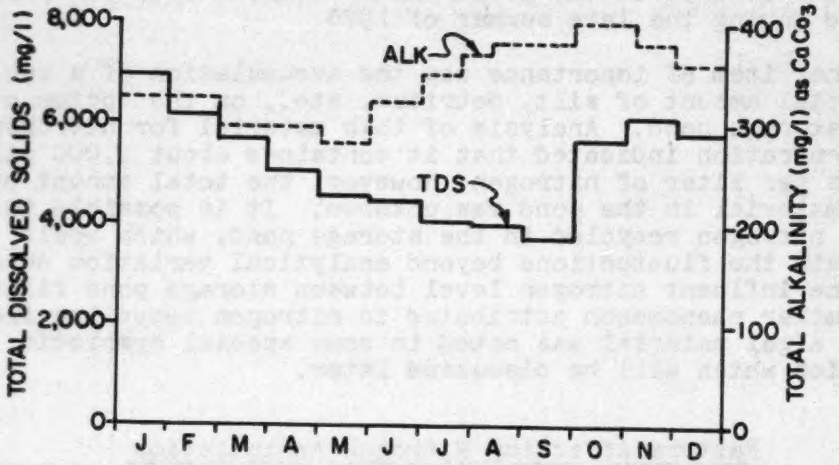

FIGURE 7 - MONTHLY VARIATION IN TOTAL DISSOLVED SOLIDS AND TOTAL ALKALINITY IN PLANT INFLUENT FOR 1970

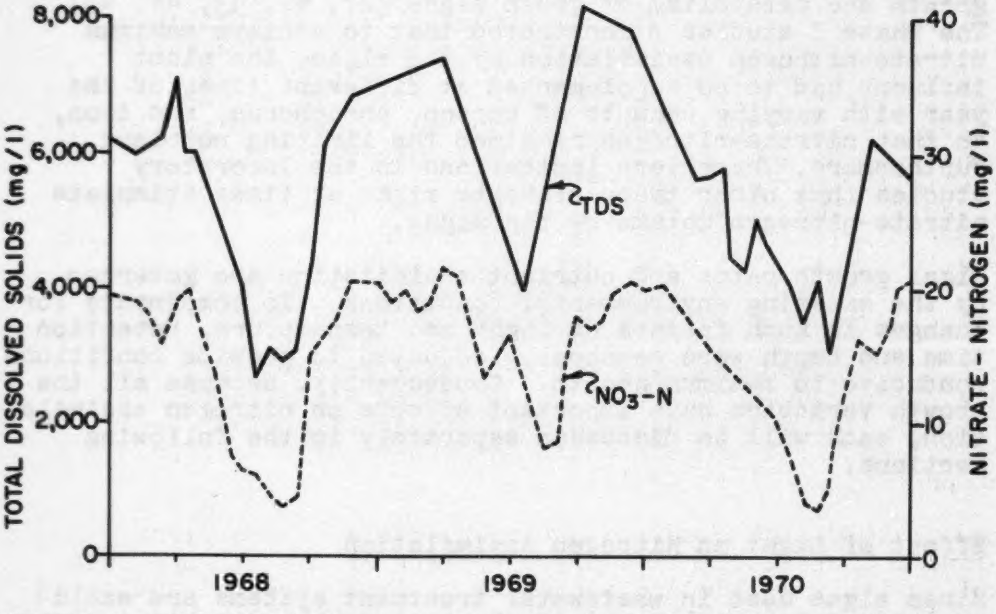

FIGURE 8 - SEASONAL VARIATION IN TOTAL DISSOLVED SOLIDS AND NITRATE-NITROGEN IN TREATMENT PLANT INFLUENT

Probably the corrosion of the storage pond roof caused a buildup of iron in the growth units without iron addition noted during the late summer of 1970.

Another item of importance was the accumulation of a substantial amount of silt, detritus, etc., on the bottom of the storage pond. Analysis of this material for nitrogen concentration indicated that it contained about 1,000 milligrams per liter of nitrogen; however, the total amount of the material in the pond was unknown. It is possible that this nitrogen recycled in the storage pond, which would explain the fluctuations beyond analytical variation noted in the influent nitrogen level between storage pond fillings. A similar phenomenon attributed to nitrogen recycling from dead algal material was noted in some special symbiotic studies which will be discussed later.

Factors Affecting Nitrogen Assimilation

According to Equation [1], carbon, nitrogen, hydrogen, oxygen, and phosphorus are the major inorganic nutrients required for production of algal cellular material. In addition, boron, B; calcium, Ca; chlorine, Cl; cobalt, Co; copper, Cu; iron, Fe; potassium, K; magnesium, Mg; manganese, Mn; molybdenum, Mo; sodium, Na; sulfur, S; vanadium, V; and zinc, Zn; are usually considered essential to normal algal growth and metabolism of green algae (27, 42, 43, 44, 45). The Phase I studies demonstrated that to achieve maximum nitrate-nitrogen assimilation by the algae, the plant influent had to be supplemented at different times of the year with varying amounts of carbon, phosphorus, and iron, so that nitrate-nitrogen remained the limiting nutrient. Furthermore, there were indications in the laboratory studies that other trace elements might at times stimulate nitrate-nitrogen uptake by the algae.

Algal growth rates and nutrient assimilation are governed by the existing environmental conditions. To compensate for changes in such factors as light and temperature, detention time and depth were seasonally adjusted to provide conditions conducive to maximum growth. Consequently, because all the growth variables have important effects on nitrogen assimilation, each will be discussed separately in the following sections.

Effect of Light on Nitrogen Assimilation

Since algae used in wastewater treatment systems are exclusively autotrophic, light can be considered to be their sole energy source (19, 32, 47). Light absorption kinetics, as related to nitrogen assimilation, have been extensively described by Shelef, Oswald, and Golueke (19), while the practical application of light factors to algal wastewater treatment systems has been described by Oswald in a number of publications (15, 17, 31, 32, 48).

Nitrogen assimilation by algae is intimately linked to photosynthesis, and several workers have shown that light in the blue wavelengths is especially favorable for nitrate reduction (33, 34). In 1920, Warburg and Negelein in a classical experiment (49) showed that light stimulated nitrate reduction by Chlorella, which is accompanied by oxygen evolution. They considered that, both in the light and dark, nitrate reduction is coupled with carbohydrate oxidation, but that in the light the carbon dioxide which might be expected as a product is assimilated by photosynthesis and replaced by oxygen evolution. Light was thought to: (1) stimulate nitrate reduction by increasing the permeability of cells to nitrate; (2) through photosynthesis, produce organic compounds available as electron donors for nitrate reduction; (3) produce a photochemical reductant; and (4) through photophosphorylation, stimulate nitrate reduction. Davis (50) found little nitrate reduction by light-limited Chlorella in the absence of carbon dioxide, unless glucose was added. He suggested that carbohydrate metabolism was necessary to form the reductant.

The absorption of light energy by dense algal cultures can be approximated by the following modification of the Beer-Lambert Law:

$$I_d = I_o e^{-Ecd} \qquad \text{Eq. [4]}$$

Where I_o is the incident light intensity, I_d is the intensity of light at any depth, d is depth in centimeters, c is the algal concentration in mg/l, E is the extinction coefficient in cm^2/mg, and e is the base of the natural logarithms. As indicated in this equation, light penetration to I_d is directly affected by incident light and inversely affected by depth and culture density. However, the Beer-Lambert Law is only valid for true solutions (33) and does not apply strictly to algal suspensions (51). Furthermore, photosynthetically linked reactions are time-intensity dependent, rather than just intensity dependent, with light utilization efficiency (growth rates) being highest at low cell densities. Gates and Borchardt (47) found that the total available light per functional cell was the important factor and that the optimal growth rate was dependent upon some minimal level of light which, if surpassed, resulted in a decrease in efficiency; and that illuminating an algal cell with more than saturating light intensities represented an inefficient use of energy. Krauss (33) found that excess light may even be detrimental to chlorophyll production. Oswald (32) stated: "The Bush equation dictates that efficiency of light use increases with depth, ... but a limit exists where the depth is so great that no light penetrates and losses, due to algal respiration, exceed their gain to photosynthesis."

The practical implication of applying light "input" to the operation of algal growth units is that if light-limiting conditions are to be avoided, the cell concentration must be adjusted so that each actively growing cell will receive optimum light. There are several ways this can be accomplished. First of all, depth and detention time can be adjusted seasonally to maximize the available light penetration; for example, long detention time-shallow depth in the winter and short detention time-deeper depths during the summer. Secondly, cell concentration can be maintained at a given level appropriate to the available light by regulation of the biomass. Another possible method of increasing the availability of light to individual cells is to move the algae into the light path by induced turbulence (mixing); however, mixing can be detrimental if nonphotosynthetic material, for example, nonassimilating older algal cells and suspended inorganic colloidal particles, interfere with light penetration. If these older cells decompose and ammonia-nitrogen becomes available under low-light conditions, it will be assimilated instead of nitrate (28). The significance of algal decomposition on nitrate assimilation will be brought out in a later section of this report.

Light availability to the algae and influent nitrogen loading were found to be the most significant variables affecting nitrogen assimilation during the Phase II investigation. The effect of light on nitrogen assimilation was measured in three miniponds of equal surface area which were operated at different depths of 8, 12, and 16 inches, and equal detention times. In addition, there were also some indications of the effect of light (depth) on nitrogen assimilation in the 1/4-acre demonstration unit, which was the only unit operated at depths of over 16 inches. During the Phase II study, this unit was operated at depths ranging from 8 to 24 inches.

The changes in the influent and effluent nitrogen concentration in units which were operated at the three depths are shown in Figure 9. From the unit startup in January until mid-April, the nitrogen removal level in the 12-inch depth unit was approximately 10 mg/l greater than in the corresponding 8- and 16-inch depth units. This was considered to be the result of insufficient light conditions, although none of the units were operating efficiently. When the carbon

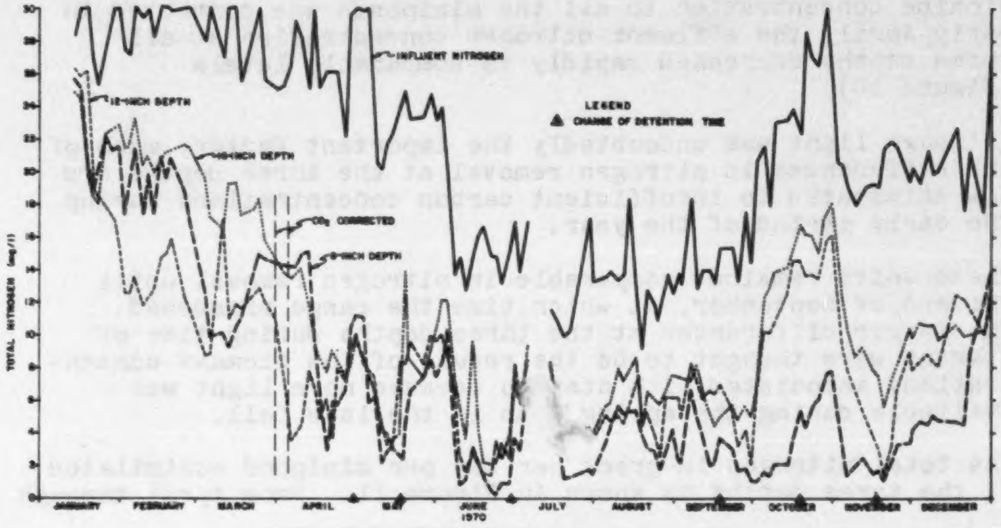

FIGURE 9 - EFFLUENT NITROGEN CONCENTRATION AS AFFECTED BY DEPTH AT MEAN DETENTION TIME

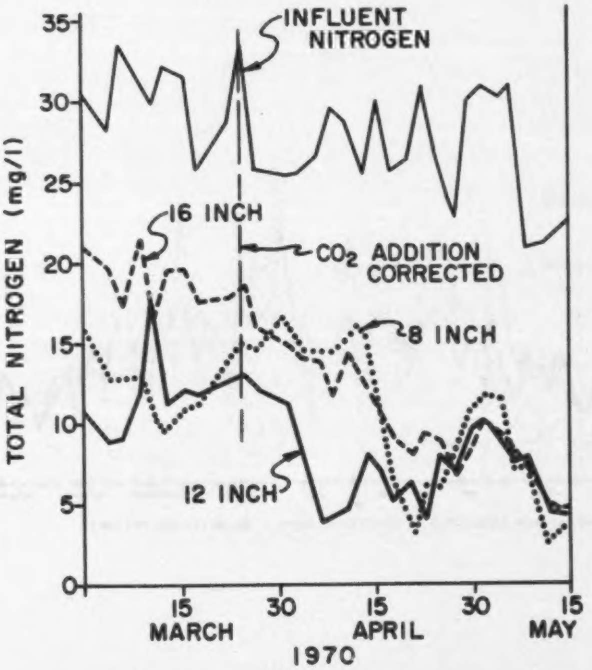

FIGURE 10- EFFECT OF CARBON DIOXIDE ADDITION ON EFFLUENT NITROGEN CONCENTRATION AT THREE DEPTHS

dioxide concentration to all the miniponds was corrected in early April, the effluent nitrogen concentration at all three depths decreased rapidly to comparable levels (Figure 10).

Although light was undoubtedly the important factor, much of the differences in nitrogen removal at the three depths are now attributed to insufficient carbon concentrations during the early period of the year.

These units remained comparable in nitrogen removal until the end of September, at which time the range broadened. The larger differences at the three depths during time of startup were thought to be the result of low biomass concentrations associated with startup because more light was available during the spring than in the late fall.

The total nitrogen in grams per day per minipond assimilated at the three depths is shown in Figure 11. From April through

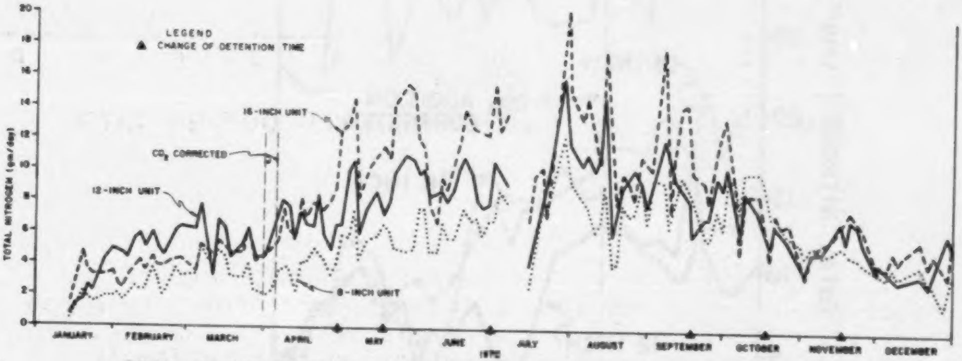

FIGURE 11 - QUANTITATIVE NITROGEN ASSIMILATION AS AFFECTED BY DEPTH - GRAMS PER DAY PER UNIT

September, the 16-inch depth unit assimilated nearly twice the nitrogen as the comparable 8-inch depth unit. Light energy calculated as total light energy per day in langleys (gm cal/cm^2/minute) is shown in Figure 12.

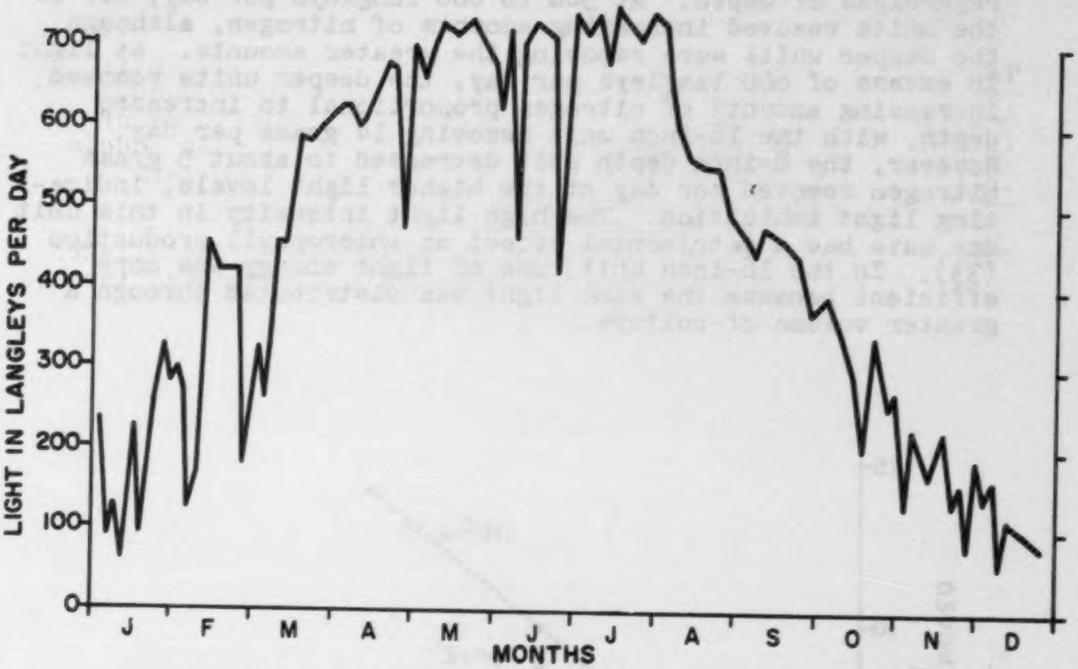

FIGURE 12 - LIGHT ENERGY RECEIVED DURING 1970

Calculations of nitrogen assimilated from April through September showed that the 16-, 12- and 8-inch depth miniponds were removing 14, 8, and 6 grams of nitrogen per day per minipond, respectively. This corresponded to a period in which light ranged from 400 to above 700 langleys per day. In October, the cultures in all three depths were removing about 8 to 10 grams of nitrogen per day and light was at about 200 to 400 langleys per day. Finally, in December, all three units averaged 5 to 6 grams nitrogen removed per day per minipond at light levels of 200 langleys or less per day.

A detailed examination of the data plotted in Figure 11 and Figure 12 shows an interesting relationship between light availability and nitrogen assimilation. The average total soluble nitrogen removed per day per minipond at the various depths during 1970 as related to the total light available is shown in Figure 13. At about 300 langleys per day, 5 grams of nitrogen were removed per day per minipond, regardless of depth. At 300 to 600 langleys per day, all of the units removed increasing amounts of nitrogen, although the deeper units were removing the greater amounts. At light in excess of 600 langleys per day, the deeper units removed increasing amounts of nitrogen proportional to increased depth, with the 16-inch unit removing 14 grams per day. However, the 8-inch depth unit decreased to about 5 grams nitrogen removed per day at the higher light levels, indicating light inhibition. The high light intensity in this unit may have had a detrimental effect on chlorophyll production (33). In the 16-inch unit, use of light energy was more efficient because the same light was distributed through a greater volume of culture.

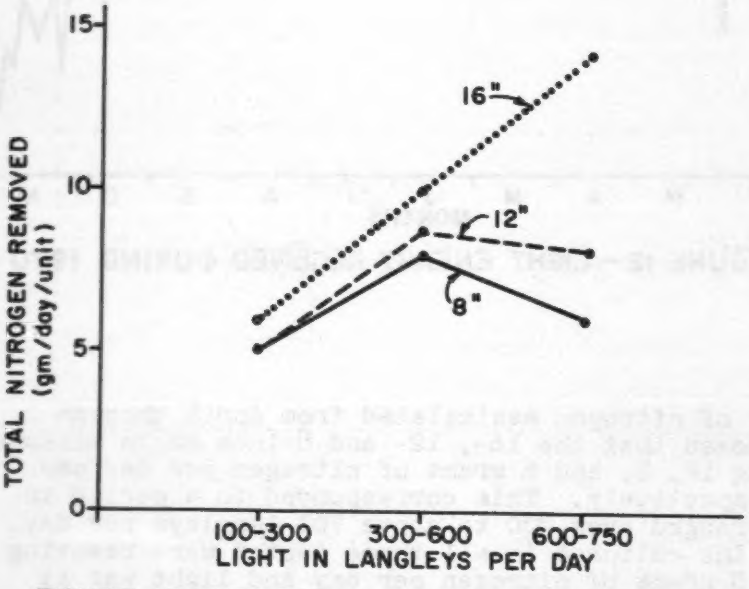

FIGURE 13 - NITROGEN REMOVAL - IN MINIPONDS AT DIFFERENT DEPTHS AND LIGHT ENERGY LEVELS.

Nitrogen assimilation in the 1/4-acre demonstration unit, operated at a 24-inch depth, is shown in Figure 14. During August, when this unit was first operated at a 24-inch depth, there apparently was enough available light (500 to 600 langleys per day) to reduce the total nitrogen in the effluent to less than 4 mg/l at a 10-day detention time. By the middle of September, the available light had diminished and the effluent nitrogen increased; however, this reduction in removal came at a time when the detention time was decreased to 8 days. Calculations indicated the effluent nitrogen would probably have remained below 5 mg/l, even at these lower light levels, if the detention time had remained constant.

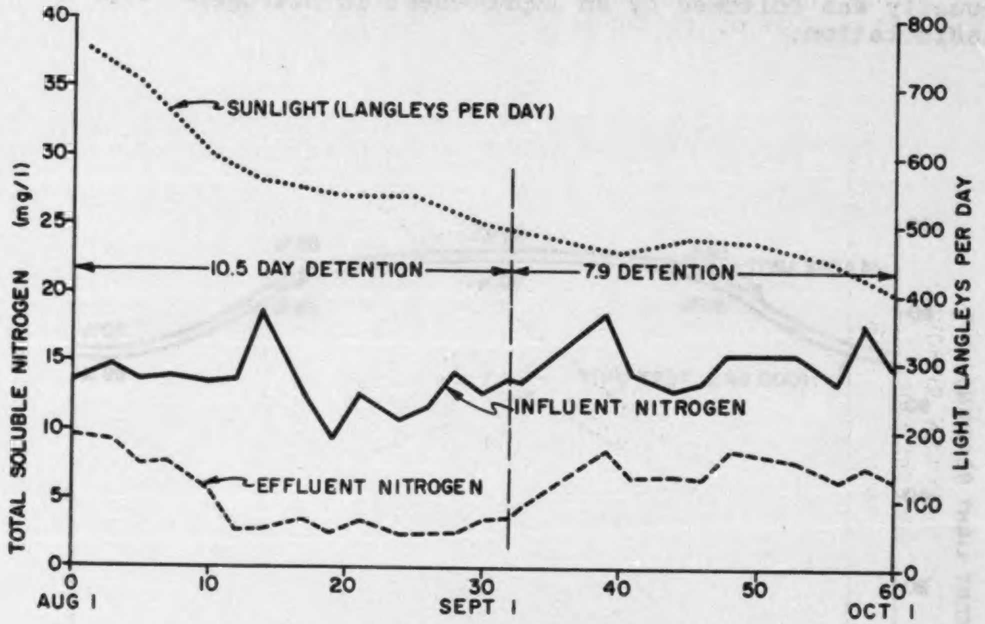

FIGURE 14 - NITROGEN ASSIMILATION IN ONE-QUARTER-ACRE UNIT AT 24-INCH DEPTH

The effect of wall shading on the actual light entering the 1/4-acre demonstration unit and a typical minipond is shown in Figure 15. The percent wall shading was calculated for a one-foot deep unit; however, the percent shading varies proportionally to the height of the divider wall and the depth and width of the unit. This wall shading was thought to be a significant factor in reducing unit efficiency, particularly during the winter months when light was critical. The effect of divider shading would, of course, become negligible as algal growth units are enlarged and shading to unit volume is reduced.

Another factor affecting light availability was shading due to algal flotation. Flotation occurred in a number of the units during the latter part of the summer in both Phases I and II, and in most cases this algal material was not removed from the surface of the unit. In the last few months of Phase II, when it became obvious that light was extremely critical, the floating algae were removed. The removal usually was followed by an improvement in nitrogen assimilation.

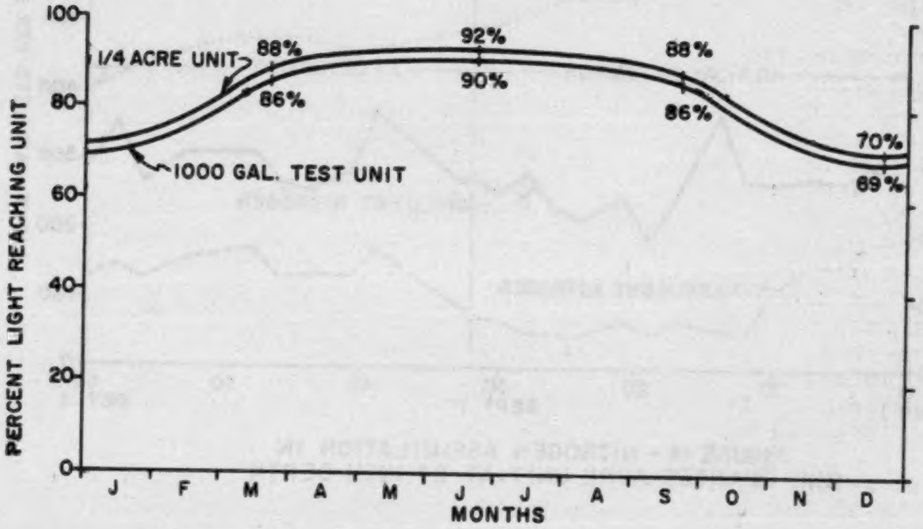

FIGURE 15 - REMAINING LIGHT DUE TO WALL SHADING

Effect of Temperature on Nitrogen Assimilation

Since temperature depends to a large extent on light intensity, in nature any change in light will also affect the growth of algae by affecting temperature (51). Accordingly, the effect of temperature on algal growth rates normally follows Van't Hoff's rule, namely a doubling for each 10°C increase in temperature within the range of temperature tolerance. Furthermore, temperature effects on growth rates have been found to be a function of light intensity; for example, Krauss (33) found that a high temperature strain of Chlorella is inhibited by light intensities above 1,000 foot-candles when grown at 25°C, but not below 3,000-foot candles at 39°C. According to Krauss, the high temperature apparently permits a higher absorption of light energy without damage. Conversely, Emerson (52) stated that the temperature at which cells are grown appears to play little part in the efficiency of photosynthesis. He found cultures grown at 10°C showed only 0.7 percent lower efficiency than cultures of corresponding density grown at 20°C and that highest efficiencies were actually observed at around 10°C. However, Oswald (53) found that the efficiency of light energy conversion by Chlorella increased linearly between 4°C and 20°C and declined above 20°C. It would then seem that though there is little doubt that temperature and light interact in their effects upon algal growth rate, the exact relationship remains controversial.

To determine the effect of temperature on algal nitrate assimilation in tile drainage, several studies were conducted with the use of the light box using shallow trays of circulating water to maintain the flask temperatures at 12.3, 21.6, and 28.0°C with a variance of ±3°C. Three extensive studies were conducted in a series during the early spring of 1970. The first study determined, among other things, that if algal cultures were started from a small inoculum, the lag and early exponential phases of algal growth were characterized by an increase in total biomass and nitrogen assimilation. These increases were a function of increased temperature, as would normally be expected; however, there were indications that once these early stages of growth were completed, the optimal temperatures for maximum nitrogen uptake decreased.

In the second experiment of the series, cultures containing an initial low cell concentration of the inoculum, mainly Scenedesmus quadricauda, were incubated at the medium temperature (21°C) until the exponential growth phase was reached, at which time some of the cultures were slowly adjusted to the extreme temperatures, 12° and 28°C, respectively. In this particular study, the general pattern of growth and nitrogen assimilation was the reverse of that noted in the first study, in that maximum nitrogen assimilation and biomass production occurred at the lowest, instead of at the highest temperature.

Finally, a third study, designed to incorporate the methods used in the first two studies, was conducted at the three temperatures (12.3, 21.6, and 28.0°C). As shown in Figure 16, during the first phase of growth, the lag period became shorter at the higher temperature. However, after this initial period, the time required for complete nitrogen assimilation was about equal at all three temperatures tested. This seems to correspond to the findings of Emerson (52). There was also a change in the predominant algal species after an extended period in the high temperature cultures that was not noted in the 12°C series. As shown in Figure 17, the changes in volatile solids in this study corresponded to changes in nitrogen assimilation rates (Figure 16).

Increases in volatile solids (Figure 17) were found to correspond directly to increases in nitrate assimilation. Furthermore, if it is assumed that each culture received the same level of light, the algae growing at the low temperatures must have been more efficient than those cells grown at high temperatures, inasmuch as the biomass production, volatile solids, and nitrate assimilation of the former were greater.

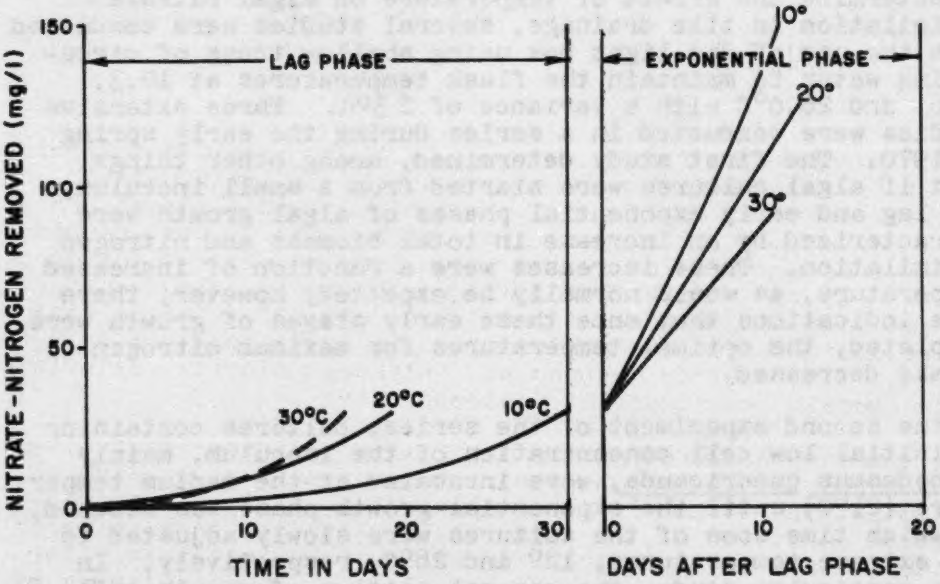

FIGURE 16 - NITROGEN ASSIMILATION AS AFFECTED BY TEMPERATURE

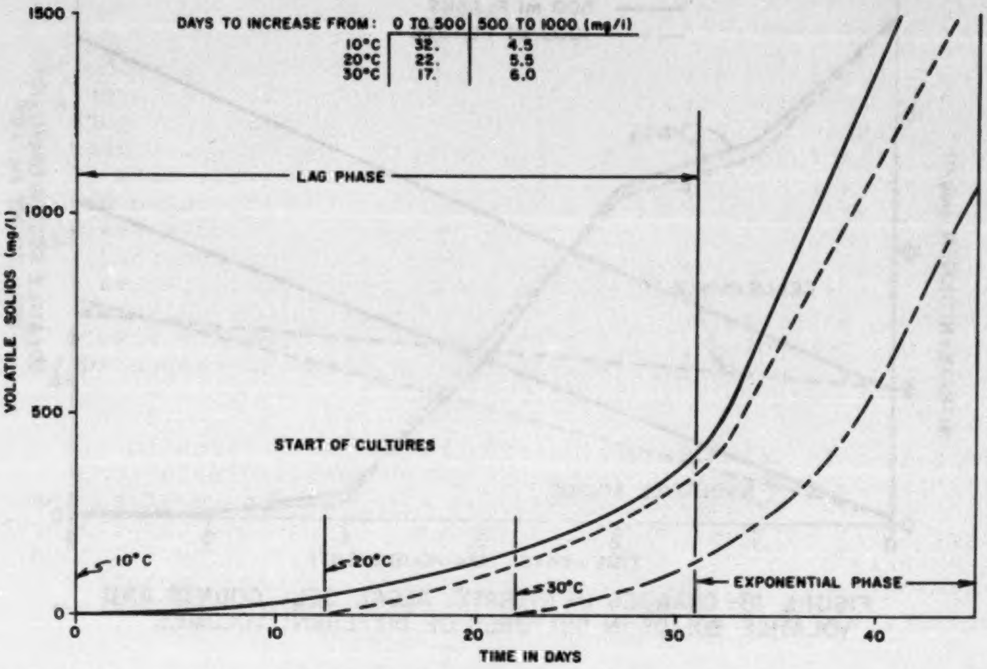

FIGURE 17-VOLATILE SOLIDS INCREASE WITH TIME AS AFFECTED BY TEMPERATURE

One other aspect of this temperature study considered significant to studies dealing with methods of analyzing algal growth potential (AGP) was the use of in vivo fluorescence as a measurement of biomass production. Use of this method of biomass measurement at the three temperatures indicated that: (1) for a given level of growth, in vivo fluorescence varied with temperature as predicted; and (2) after initial correlation to nitrate assimilation in early growth stages, in vivo fluorescence dropped off, although nitrate assimilation (growth) continued at a steady rate.

The studies conducted at the IAWTC also indicated that biomass production is not necessarily synonymous with nitrogen assimilation, within limits. Apparently, it is the physiological condition of the algae rather than the total biomass produced that is significant in nitrogen assimilation. For example, in many of the light box studies, algal cultures of one-half the density of other cultures were found to assimilate more or equal amounts of nitrogen (Figure 18).

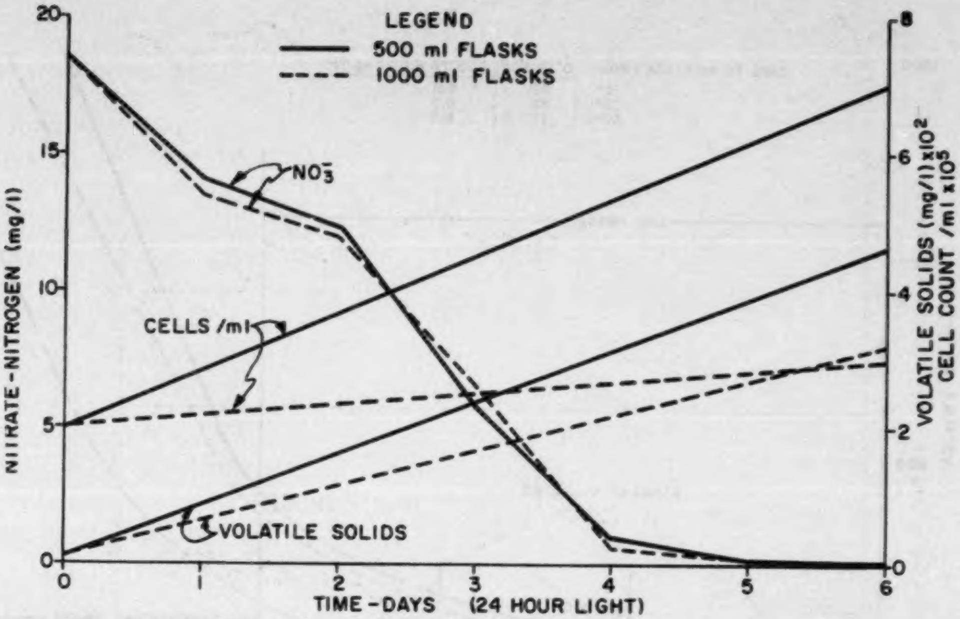

FIGURE 18- CHANGES IN NITRATE, ALGAL CELL COUNTS AND VOLATILE SOLIDS IN CULTURES OF DIFFERENT VOLUMES

A special temperature study conducted with the use of a 1,000-gallon minipond in the fall of 1970 (Figure 19) indicated that light had a greater effect than temperature on nitrogen assimilation. A comparison of nitrogen assimilation by an algal culture grown in a unit at ambient temperature and with that of one grown at summer temperatures of 25-30°C showed that the higher temperature had no beneficial effect on nitrogen assimilation.

Flask bioassays seemed to indicate that high temperature, rather than excess light, was detrimental to the algal system under study, although under certain circumstances both would be equally inhibitory. High temperature probably affected the system in several ways: (1) by directly inhibiting the algae, (2) by increasing the nitrogen in the system via speeded up sludge decomposition and resulting nitrogen regeneration, and (3) by adversely affecting nutrient solubility.

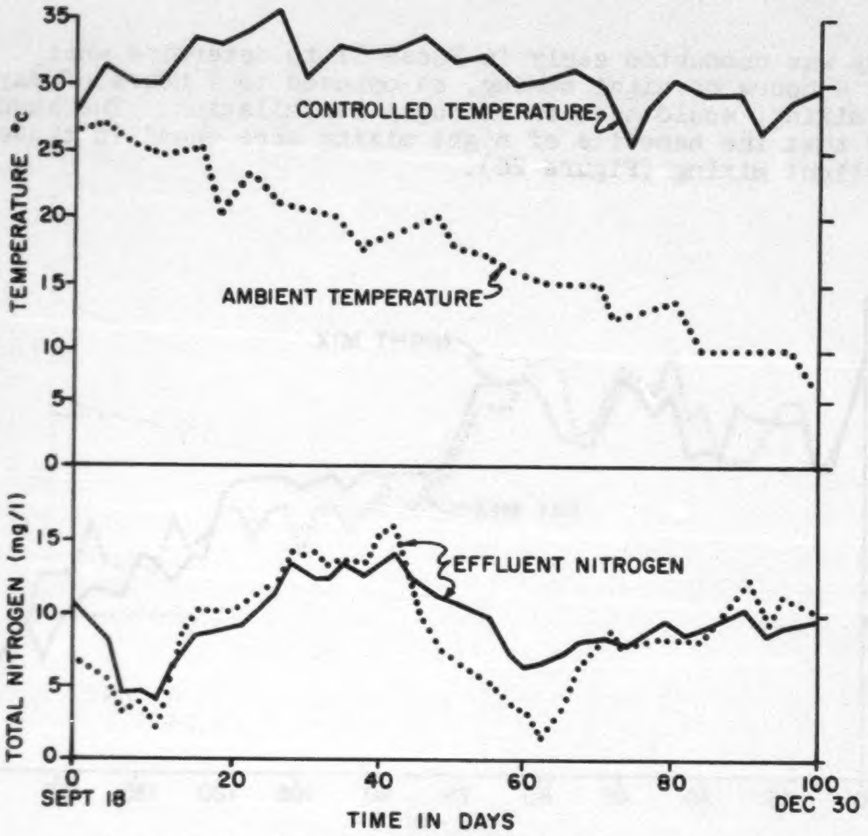

FIGURE 19- EFFECT OF TEMPERATURE ON ALGAL NITROGEN ASSIMILATION

Effect of Mixing on Nitrogen Assimilation

The Phase I studies showed that mixing can affect algal systems in a number of ways: (1) by moving the algae into the light zone; (2) by reducing the extent of the anaerobic areas; (3) by removing the solid material from the system by keeping the solids in suspension and available for discharge in the effluent; (4) by replenishing carbon dioxide exchange from the air to increase the surface area exposed to the atmosphere; (5) by stirring the bottom deposits, thus making more nutrients available; and (6) by preventing thermal stratification. A study on the effect of duration of mixing early in the Phase I investigation showed that a four-hour period of daylight mix was optimal for maximum nitrogen assimilation. However, the study did not include a strictly night mix regime, although 24-hour mixing was included.

A study was conducted early in Phase II to determine what effect 4 hours of night mixing, as opposed to 4 hours of daylight mixing, would have on nitrogen assimilation. The study showed that the benefits of night mixing were equal to those of daylight mixing (Figure 20).

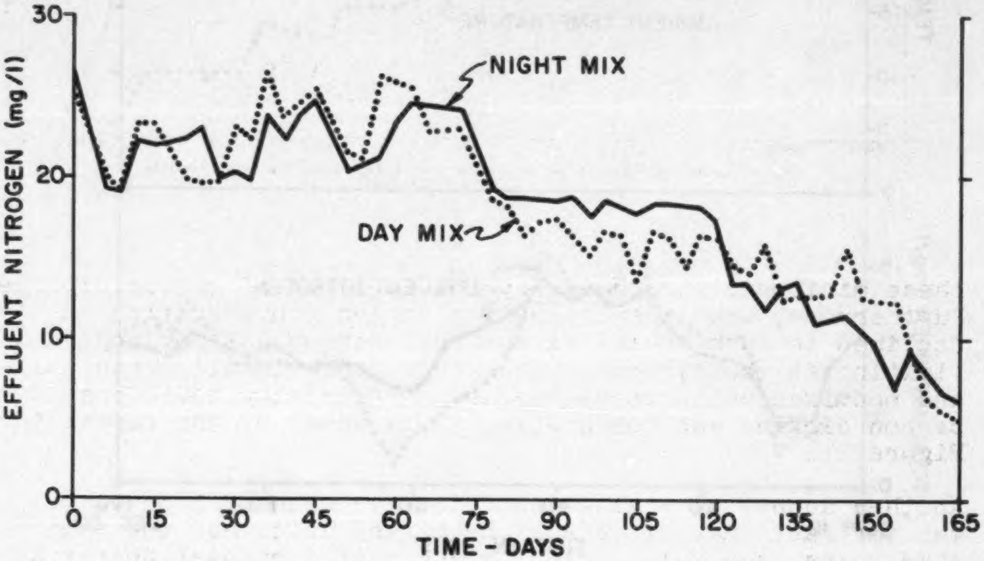

FIGURE 20-EFFECT OF DAY OR NIGHT MIXING ON NITROGEN ASSIMILATION

When the performance of cultures under day mixing, with and without the addition of carbon dioxide, was compared to that of a nonmixed unit receiving no carbon addition, very little difference in nitrogen assimilation was observed between those mixed and nonmixed units which did not receive added carbon dioxide. To test the hypothesis that carbon addition and not mixing was the limiting factor affecting nitrogen assimilation by the algae, one test unit was operated on a nonmix schedule and carbon dioxide was injected by means of a diffuser. Algal nitrogen assimilation in this unit was then compared to that occurring in mixed units, with and without carbon dioxide addition, and a nonmixed, no-carbon-dioxide unit (Figure 21). During the first few months, the only difference observed in nitrate assimilation between

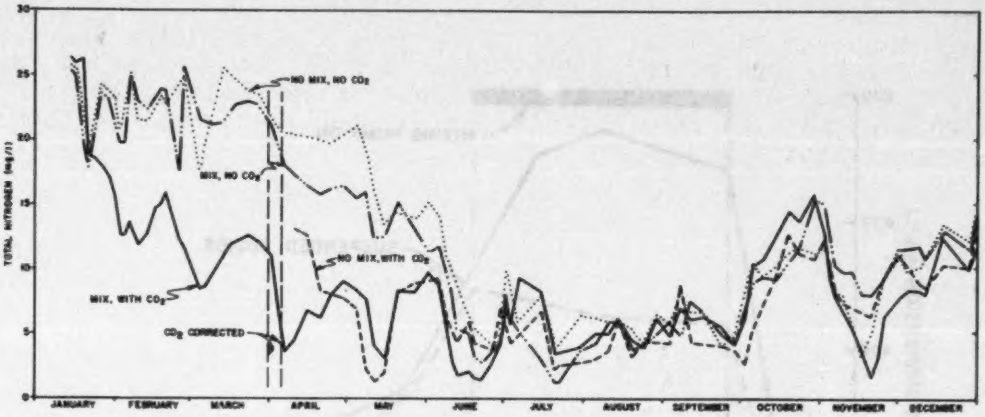

FIGURE 21-EFFECT OF MIXING AND NO MIXING WITH AND WITHOUT ADDITION OF CARBON DIOXIDE ON NITROGEN ASSIMILATION

these miniponds was caused by the addition of carbon dioxide. Furthermore, when the influent nitrogen concentration declined in June to the extent that carbon was no longer limiting in the systems, nitrogen removal in all of the mixed and nonmixed units receiving or not receiving additional carbon dioxide was comparable, as is shown by the curves in Figure 21.

Another aspect of mixing found to have been of importance was the fact that since all the mixing units had the same size pumps, the water velocity and solids removal became a function of pond depth. A measurement of velocities within the individual units showed a wide range between units. In addition, since the effluent had to pass out of a riser-tube arrangement and flow velocities were often negligible, material of greater density than water tended to stay in the miniponds (Figure 6). The change in concentration of volatile and suspended solids in the pond during a mixing cycle is plotted in Figure 22, which shows that the suspended solids began to settle even before the mixing pump had been turned off. Mixing at velocities to 0.5 foot per second was not found to be adequate to remove much of the settleable solids in the unit. As a result, a sludge buildup occurred as time progressed. These differences in pumping per unit-volume could not be conveniently corrected during the study and probably were a factor in reducing the efficiency of the deeper units.

The results of Phase II studies, although indirect, indicate that in spite of any beneficial effect it may have, mixing of an algal growth unit containing large quantities of sludge may be detrimental. One possibility is that mixing brings

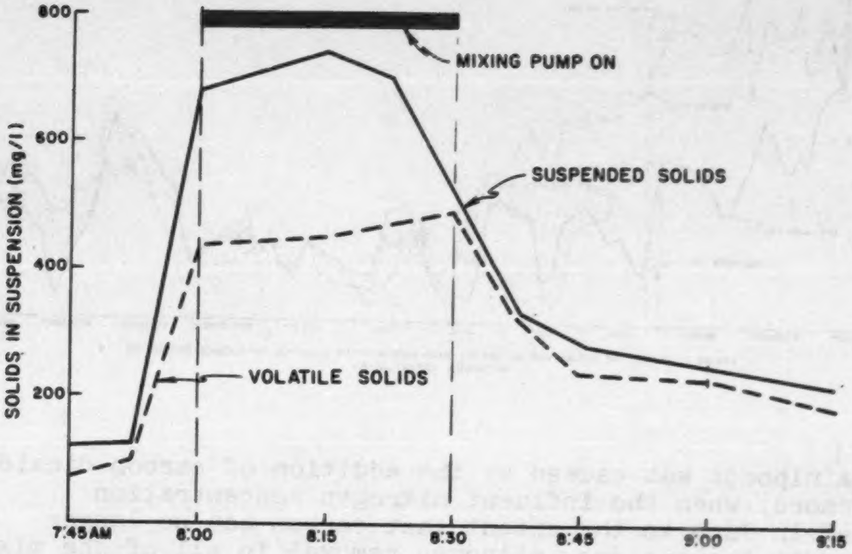

FIGURE 22. SUSPENSION OF SOLIDS DURING MIXING

nonphotosynthesizing material into suspension, which reduces the available light per active cell. A second reason is that mixing can lead to the sludge becoming aerobic, which in turn may result in bacterial nitrogen fixation, possibly adding to the total nitrogen in the system. Conversely, if the system is not mixed, the sludge becomes anaerobic and as a consequence some of the nitrogen in the algal system is removed through bacterial assimilation and/or denitrification. Thirdly, by stirring the sludge, nutrients become less available because of the precipitation of phosphate and iron which usually occurs when the sludge becomes aerobic.

Effect of Detention Time on Nitrogen Assimilation

Figure 23 shows the change in the influent and effluent total nitrogen during 1970 in 12- and 8-inch units operated at three different detention times, with and without carbon dioxide addition. From the early part of the year through September, there was about 3-5 mg/l difference in nitrogen assimilation at the three detention times tested, although the longer detention time units did tend to be accompanied by slightly lower (1-2 mg/l) effluent nitrogen concentration

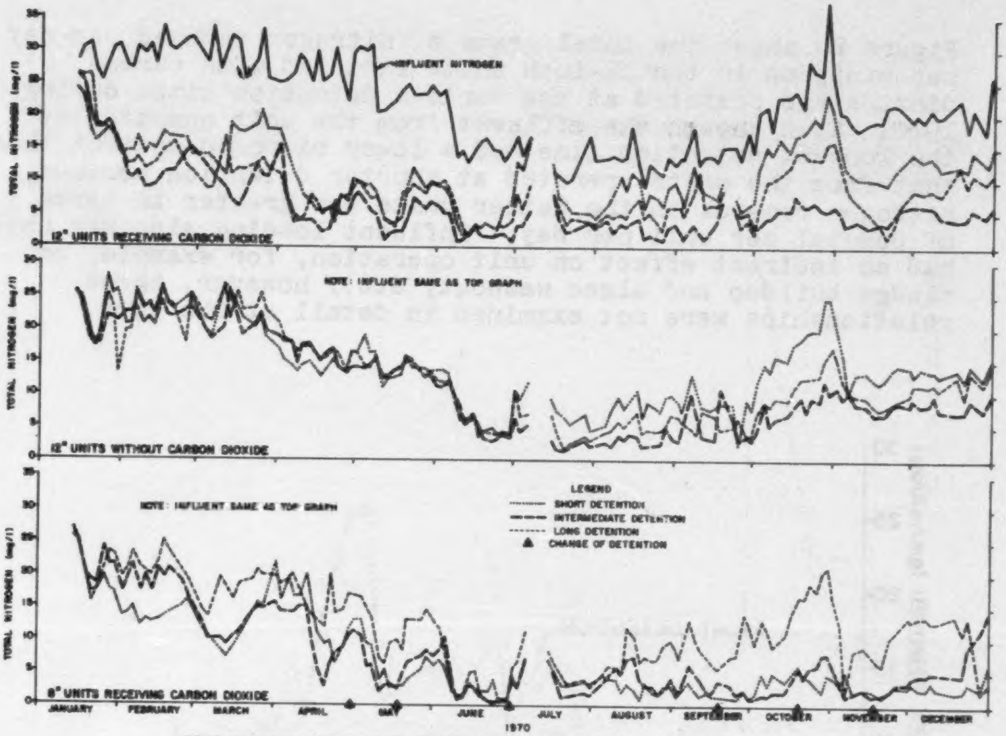

FIGURE 23-EFFECT OF DETENTION ON NITROGEN ASSIMILATION IN 8-INCH AND 12-INCH UNITS

than were the intermediate and short detention times. From the early part of the year through September, detention times ranged from as long as 16 days in January to as short as 1 day in July. Usually, the spread between the short and long detention time periods was on the order of 2 to 5 detention times, depending on time of the year. Because detention time is a major operational parameter in algal growth systems, some other factor must have limited nitrogen assimilation by the algae.

Late in the fall, the differences in nitrogen removal between the three detention time units increased. During this period, algal growth rates were quite low due to limited light conditions and detention times apparently were adequate to bracket the optimal flow required for maximum nitrogen removal.

Figure 24 shows the total grams of nitrogen removed per day per minipond in the 12-inch units enriched with carbon dioxide and operated at the various detention times during 1970. Even though the effluent from the unit operated at the longest detention time had a lower nitrogen content than that from the units operated at shorter detention periods, nitrogen removal in the latter units was greater in terms of removal per unit per day. Influent loading also may have had an indirect effect on unit operation, for example, on sludge buildup and algae washout, etc.; however, these relationships were not examined in detail at the time.

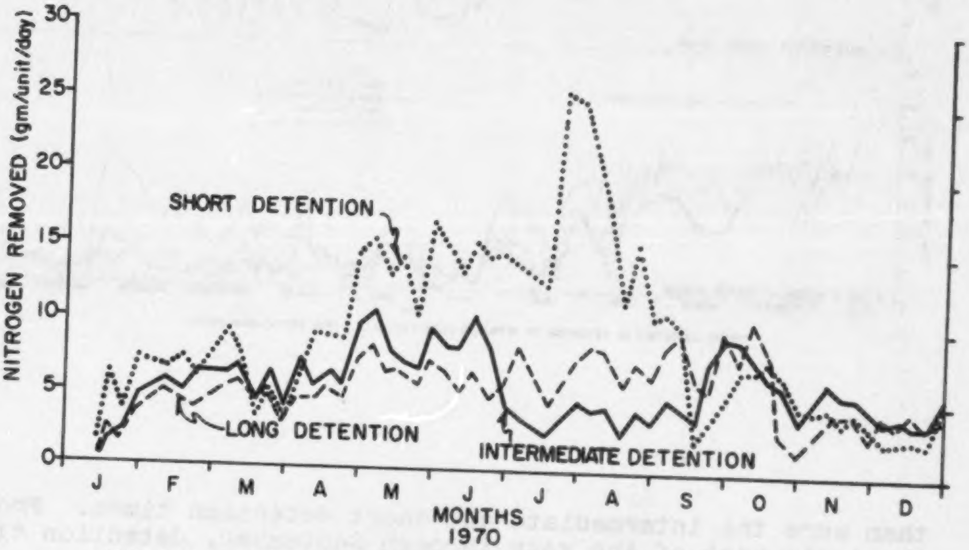

FIGURE 24-NITROGEN REMOVED AT DIFFERENT DETENTION TIMES AT 12 INCH DEPTH WITH CO_2

Effect of Carbon on Nitrogen Assimilation

Autotrophic photosynthesizing algae, unlike heterotrophic bacteria and algae, use inorganic carbon as their carbon source. Carbon dioxide is the most common form of carbon used (33), although Scenedesmus, the predominant species studied at the IAWTC, and other algae supposedly can utilize bicarbonate as readily as carbon dioxide (54). Regardless of the carbon form used, there is considerable disagreement as to the form in which it actually penetrates the cell or chloroplast (33).

Natural oligotrophic bodies of water probably contain an almost limitless supply of inorganic carbon for algae growth (47) in the form of alkalinity and from carbon dioxide absorption from the atmosphere. However, Gotaas et al (55) found that the lack of sufficient inorganic carbon in raw sewage can limit the production of algae. The same is probably true of any water rich in nutrients but poor in carbon.

In poorly buffered systems, the assimilation of carbon dioxide and bicarbonate by actively growing algae causes the equilibrium indicated in the following equation to shift to the right and the pH to rise.

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^- \longleftrightarrow H^+ + CO_3^= \qquad \text{Eq. [5]}$$

The concentration of any of the components of the carbon dioxide-bicarbonate-carbonate buffer system is a function of the temperature, pH, and TDS, as well as of the concentration of the remaining nutrient components (56, 57). The equilibrium equation for the formation of hydrogen and bicarbonate ions from carbonic acid is:

$$\frac{(HCO_3^-)(H^+)}{(H_2CO_3)} = K_1 \qquad \text{Eq. [6]}$$

In the Handbook of Chemistry and Physics (58), the dissociation constant, K_1, is reported to be 3.5×10^{-7} at 18°C. At a pH of 8 the ratio of carbonic acid to bicarbonate ion is 0.0286, at a pH of 7 it is 0.286, and at a pH of 6 it is 2.86 (59). A similar equilibrium reaction between bicarbonate and carbon dioxide has a reported K_2 of 4.4×10^{-11} at 25°C (58); thus at pH 7, the ratio of bicarbonate to carbonate ions would be 2,270 to 1, whereas at pH 11 the ratio would be 1 to 4.4 (88).

At pH values above 9, carbonate precipitates as calcium and magnesium salts, thus decreasing the total alkalinity. Furthermore, these precipitates also remove many algal nutrients, especially phosphorus and heavy-metal trace elements. This precipitation of nutrients with increase in pH was found to be a significant factor in the operation of the IAWTC algal test units, in which the pH levels were often over 10.

Nitrate reduction by algae is very dependent upon the products of photosynthesis (34). Photosynthesis produces carbohydrates which in turn provide the hydrogen donors required for nitrate reduction. Consequently, green algae in the absence of carbon dioxide are usually unable to reduce nitrate-nitrogen at a high rate. Davis (50) found that a ten-fold increase in nitrate reduction in the light would take place after adding a carbon source. Bongers (24) was able to inhibit the incorporation of ammonia (the final step in the reduction process) into amino acids by limiting the supply of carbon dioxide in the presence of light. Bongers also found that cells grown in a complete nutrient medium (nitrogen content 8 to 10 percent) in light, under carbon dioxide-deficient conditions, excrete an amount of ammonia into the medium equal to the amount of nitrate disappearing from the medium. This was thought to be the result of a lack of suitable carbon skeletons to function as ammonia acceptors. Kessler (34) found that as the carbohydrate reserves of a cell are exhausted, there is a considerable increase in nitrite accumulation in the medium, and presumably nitrate reduction continues (50), even though nitrite and ammonia are not assimilated. Furthermore, ammonia-nitrogen has been reported to be toxic at pH values above 9 (24).

Several workers (53, 60) have reported that concentrations of carbon dioxide much in excess of 0.5 to 10 percent are either toxic to algae or are growth-rate limiting. Conversely, Tew _et al_ (38) and Gates and Borchardt (47) have been able to grow algae at concentrations of carbon dioxide as high as 100 percent. Tew _et al_ concluded that it is feasible to use highly concentrated carbon dioxide for continuous algal growth, if the growth rate is balanced with carbon dioxide addition rates. Since many of the earlier workers did not account for a total balanced nutrient system as a function of carbon dioxide addition, this might explain their inability to use carbon dioxide levels higher than 10 percent.

One other facet of carbon dioxide addition pertinent to the present study is the effect it has on the pH of the growth medium. Changes of pH in the medium is a function of algal cell growth (photosynthesis); the pH rises as carbon and nitrate are assimilated by actively growing cells. As the pH increases, many of the nutrients necessary for growth precipitate out of solution (28). In the precipitated state, their availability to algal growth is questionable. The addition of supplemental carbon dioxide helps to stabilize pH in an actively growing culture, acting both as a carbon source and, by lowering the pH level, as a means of maintaining nutrients in solution.

Since algae are approximately 50 percent carbon and 8 to 10 percent nitrogen, theoretically the ratio of carbon to nitrogen in the growth medium should be 5:1. Comparison of the influent total alkalinities with predicted nitrogen concentration (Figures 3 and 7) indicates that carbon probably would be a limiting nutrient during those times of the year when the nitrogen content of the influent is high;

however, these calculations did not take into consideration any air-water carbon dioxide exchange.

Because the relationship of carbon to nitrogen requirements in tile drainage was unknown, a number of light box studies were conducted to determine the effect of carbon addition on nitrogen assimilation. In general, the studies showed that the amount of carbon available to the algae during nitrogen assimilation was an important factor. Data plotted in Figures 25 and 26 from studies by Brown and Arthur (41) indicate the typical response of algal nitrogen assimilation and biomass production to the addition of various concentrations of carbon.

In several other studies, the effect of carbon addition as bicarbonate (a form thought to be utilized by Scenedesmus) on nitrate assimilation was compared to that of 4 percent carbon dioxide addition. The tests seemed to indicate that bicarbonate-carbon, injected as sodium bicarbonate, was not available for growth of Scenedesmus quadricauda. Studies conducted later with the use of a minipond also indicated that this form of carbon addition may not be as available as that of carbon dioxide to Scenedesmus quadricauda. Initially, when sodium bicarbonate was added to this minipond there was a positive response by the Scenedesmus quadricauda culture in both appearance and nitrogen assimilation. However, within a 2- to 3-week period, the Scenedesmus culture was replaced by a variety of green and blue-green algae. An examination of the minipond showed a large amount (1-2 inches) of sludge, which was assumed to be carbonate compounds. Nevertheless, even though there were different algae in this unit, nitrogen removal continued to be comparable to a minipond which was receiving carbon dioxide for the remainder of the year. The initial amount of sodium bicarbonate added to this unit appears to have been too great.

Early in the Phase II study, it was decided to determine whether the carbon-to-nitrogen ratio in the tile drainage could be maintained by using the carbon available in the alkalinity as a basis for calculating the carbon addition required. Since the pH in the tile drainage was usually about 7.0 to 7.5, most of the carbon was available as bicarbonate. The carbon addition was estimated as follows:

$$C_a = 5N_i - C_i \qquad \text{Eq. [7]}$$

Where C_a = carbon addition required, N_i = influent nitrogen in mg/l-N, and C_i = influent carbon from bicarbonate alkalinity in mg/l-C.

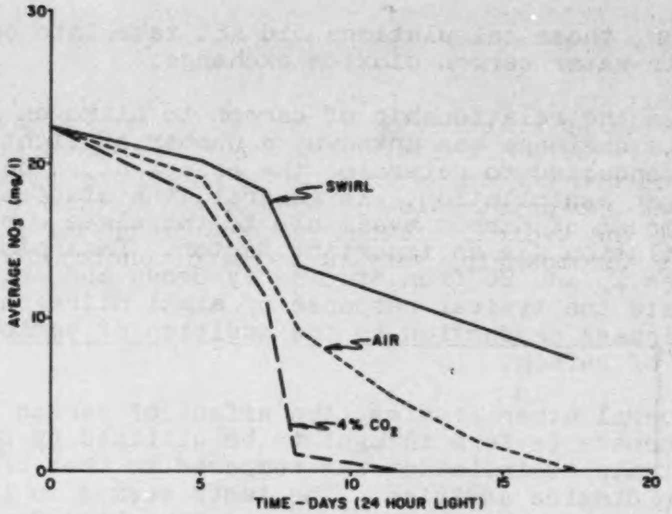

FIGURE 25 – THE EFFECT OF VARIOUS LEVELS OF AERATION ON NITRATE REMOVAL IN THE LIGHT BOX

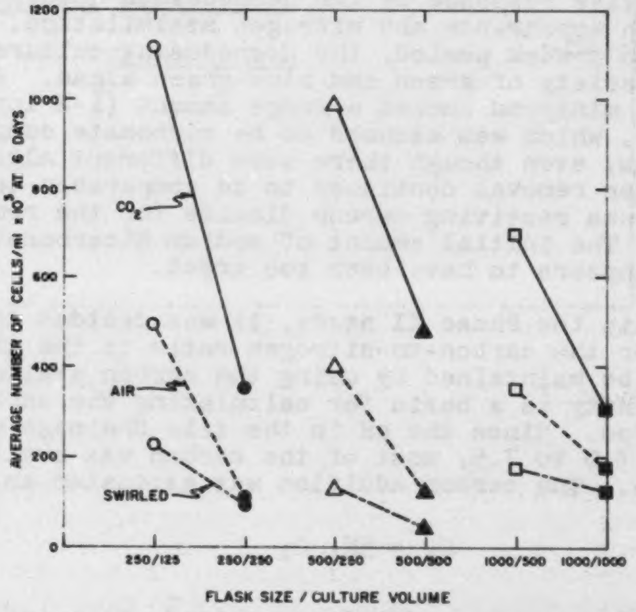

FIGURE 26 – EFFECT OF SURFACE/VOLUME RELATIONSHIP AND VARIOUS TYPES OF AERATION ON CELL NUMBERS AT SIX DAYS, AVERAGE OF FIVE REPLICATIONS

FROM BROWN AND ARTHUR (41)

Although this method of estimating the required carbon did not take into account such things as (1) actual carbon availability, (2) carbon dioxide air-water exchange, and (3) calcium carbonate precipitation at high pH values, it did serve as an effective way of estimating the amount of carbon required in the units. The studies further showed that carbon had to be injected only during afternoon periods of peak photosynthesis. The data as plotted in Figure 27 depict the typical diurnal changes in pH and bicarbonate levels accompanying varying rates of photosynthesis.

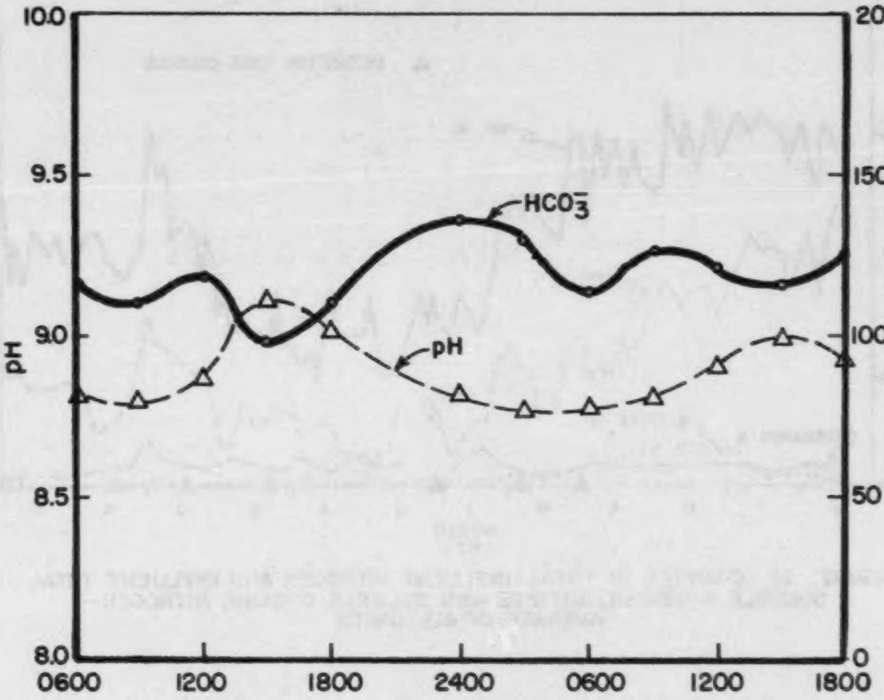

Figure 27 - TYPICAL SHIFT IN pH AND HCO_3^- IN ALGAL TEST UNITS

Actual operation of the units showed that there were more problems to be encountered in injecting the carbon dioxide into the system in the correct amount than in determining the correct carbon concentration. Automatic carbon dioxide injection with pH control would probably have eliminated many of the operational problems encountered at the IAWTC.

Figure 28 shows the average concentration of the total influent and effluent nitrogen. It also depicts the average effluent organic and nitrite-nitrogen concentrations during 1970. During the period January through May, the nitrite concentration in most of the units was abnormally high, with several miniponds over 5 mg/l nitrite-nitrogen.

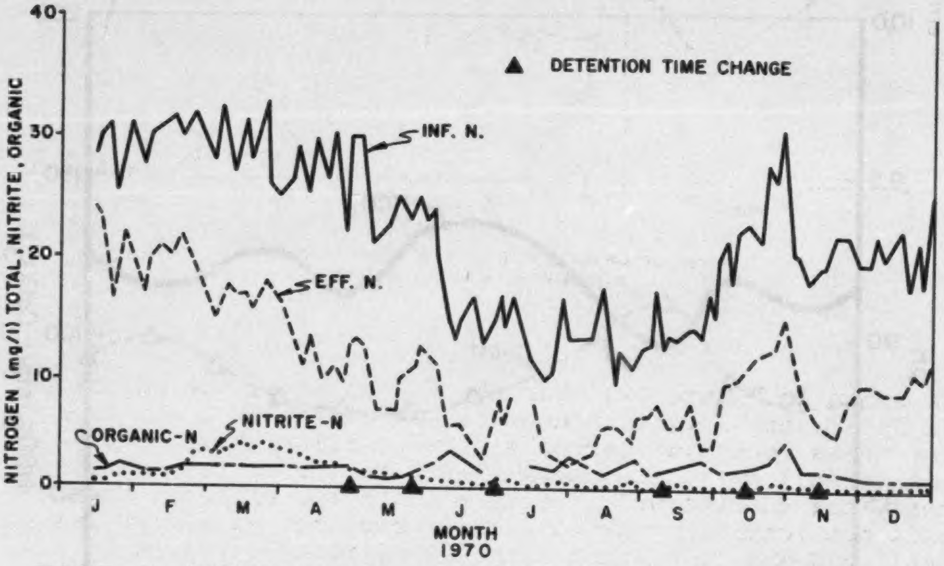

FIGURE 28 - CHANGES IN TOTAL INFLUENT NITROGEN AND EFFLUENT TOTAL SOLUBLE NITROGEN, NITRITE AND SOLUBLE ORGANIC NITROGEN - AVERAGE OF ALL UNITS

Since virtually no nitrite entered the system by way of the influent, it appeared that nitrate was not being completely reduced and, as a result, nitrite was being released into the culture medium. A comparison of the performance of a 12-inch unit with and without carbon dioxide addition (Figure 29) indicated that this nitrite release probably was the result of a carbon deficiency for the amount of nitrogen to be assimilated. This result agrees with the finding of Krauss (33).

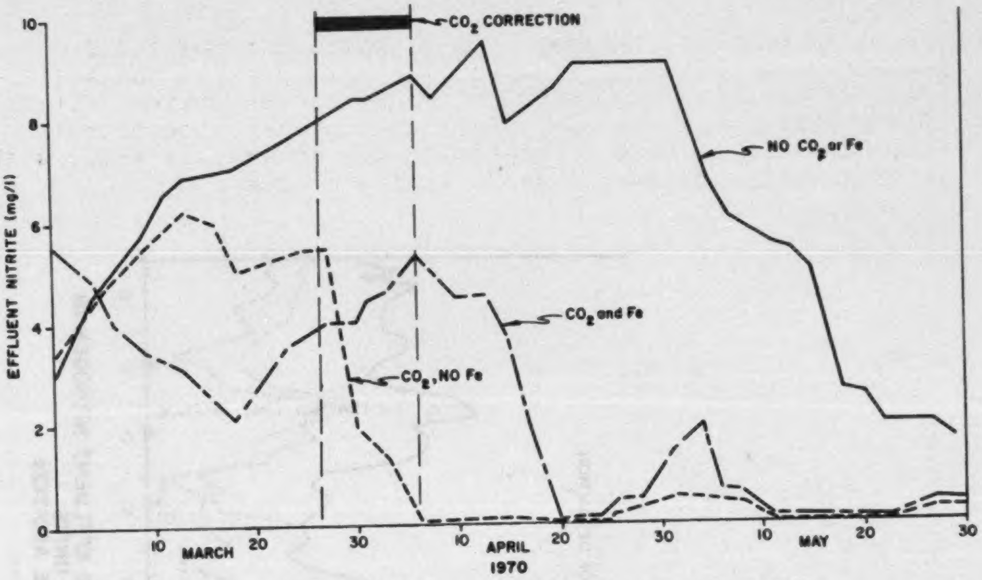

FIGURE 29. CHANGES IN NITRITE CONCENTRATION IN TEST UNITS WITH AND WITHOUT CO_2 ADDITION

As shown in Figure 29, when the carbon level was adjusted in April, the nitrite concentration of the effluent from the units receiving carbon dioxide declined immediately to less than 1 mg/l, the normal level in the system. Furthermore, the concentration of nitrite in the effluent from the unit not receiving carbon dioxide remained at a high level until mid-May, at which time the nitrite decreased in response to the decline in the carbon requirement. After this time, the nitrite concentration of the effluent from all the test units continued at a low level for the remainder of the year (Figure 28).

The above data seem to indicate that, under conditions of carbon deficiency, nitrate can be taken into the cell but cannot be completely reduced to ammonia, and that nitrite is then released from the cell at a level proportional to the carbon deficiency. One other possibility is that bacterial denitrification was occurring in the test units at a fairly high rate and that the carbon deficiency affected their rate of nitrate reduction. In any case, if this carbon deficiency had been recognized earlier in the year, measures could have been taken to improve the nitrogen removal efficiencies of the test units (Figure 30).

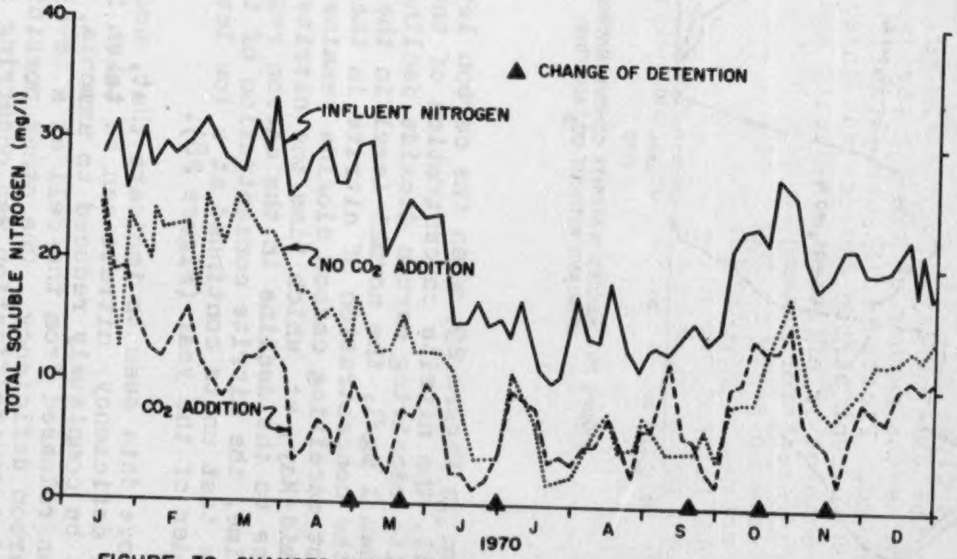

FIGURE 30-CHANGES IN TOTAL INFLUENT AND EFFLUENT NITROGEN IN INTERMEDIATE DETENTION TIME UNITS WITH AND WITHOUT CARBON DIOXIDE ADDITION

In August and September 1970, the culture in a minipond was maintained at a pH of 9.0±.5 by injection of controlled amounts of 100-percent carbon dioxide into the unit. The carbon dioxide injection device consisted of an "in pond" pH probe connected to a regulatory unit by which the desired pH could be maintained by the opening and closing of a solenoid valve on a 100 percent carbon dioxide cylinder. The gas was injected into the culture through a diffuser system placed on the bottom of the test unit containing the culture. A clock was then connected to the solenoid to indicate the time of day at which the pH exceeded 9.0 and to determine the number of minutes per day carbon dioxide was injected. Plotted in Figure 31 are the minutes during which 100 percent carbon dioxide was injected each day, and the changes in effluent alkalinity and nitrogen. With this arrangement for automatic pH control, the carbon dioxide came on early in the evening, much later than had been expected. A hypothesis for the lateness of the hour of high pH is that the stored cellular carbohydrates were being used prior to peak photosynthesis. The carbon addition which followed this peak served to replenish the carbohydrate level. Thus, it is possible that carbon dioxide was being injected prematurely in the rest of the units during 1970 as they received carbon dioxide from 1:00 to 4:00 p.m. each day.

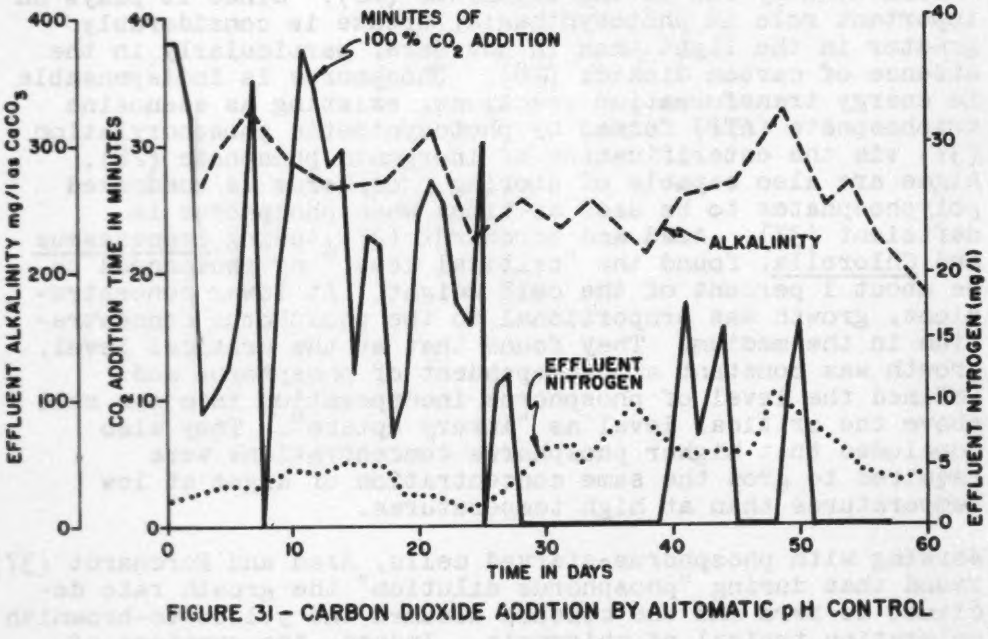

FIGURE 31 - CARBON DIOXIDE ADDITION BY AUTOMATIC pH CONTROL

The changes in length of time of 100 percent carbon dioxide injection per day indicated the existence of a period of carbon dioxide utilization which was always followed by a recovery period during which the pH stayed below 9.0. Apparently, the only thing saving the unit from complete failure was the self-regulating pH mechanism. Possibly because the gas was injected as very large bubbles, some algal cells came into immediate contact with 100 percent carbon dioxide gas which was toxic to the cells. The possible toxicity of 100 percent carbon dioxide injection to algae and its inhibition of nitrogen removal were discussed in the Phase I report.

Later, the 100 percent carbon dioxide concentration was reduced to 4 percent to determine whether carbon dioxide concentration influenced algal response. However, the unit was not operated at a time when the carbon supply was inadequate. Consequently, the results are inconclusive in terms of effect of carbon dioxide concentration.

Effect of Phosphorus and Iron on Nitrogen Assimilation

Phosphorus is one of the major nutrients required for the normal growth of algae and is frequently a limiting factor of algal growth in nature (22, 42, 61). In solution, phosphorus is primarily present as inorganic orthophosphate, a form used by all living organisms (62). Since it plays an important role in photosynthesis, uptake is considerably greater in the light than in the dark, particularly in the absence of carbon dioxide (28). Phosphorus is indispensable in energy transformation reactions, existing as adenosine triphosphate (ATP) formed by photosynthetic phosphorylation (37) via the esterification of inorganic phosphate (28). Algae are also capable of storing phosphorus as condensed polyphosphates to be used at times when phosphorus is deficient (37). Azad and Borchardt (37), using *Scenedesmus* and *Chlorella*, found the "critical level" of phosphorus to be about 1 percent of the cell weight. At lower concentrations, growth was proportional to the phosphorus concentration in the medium. They found that at the critical level, growth was constant and independent of phosphorus and defined the level of phosphorus incorporation into the cell above the critical level as "luxury uptake". They also concluded that higher phosphorus concentrations were required to grow the same concentration of algae at low temperatures than at high temperatures.

Working with phosphorus-starved cells, Azad and Borchardt (37) found that during "phosphorus dilution" the growth rate declined to zero and the culture assumed the yellow-to-brownish coloration typical of chlorosis. Indeed, the symptoms of phosphorus deficiency are the accumulation of fat, starch, and cell wall substance which indicates some interference with nitrogen metabolism (28).

Stumm and Leckie (22), discussing natural bodies of water, stated that it is not possible to establish a critical level of phosphorus because the rate of biomass production is primarily influenced by the rate of supply of soluble phosphorus to the algae. According to them, the rate is function of: (1) regeneration of nutrients from the biota and detritus, (2) the supply in the influent, (3) the exchange with the sediments, and (4) the transport process (diffusion).

The pH of the medium may alter the rate of phosphorus uptake either by a direct effect on permeability of the cell membrane or by changing the ionic form of the phosphate (28). As the pH level rises due to the activity of growing algal cells, inorganic solid phases of phosphorus may form by direct precipitation of phosphorus with calcium, aluminum, and iron compounds as well as with clays (21, 22, 26, 37, 51, 63, 64). Figure 32, taken from a paper by Stumm and Leckie (22), depicts the solubility of different phosphate phases as a function of pH.

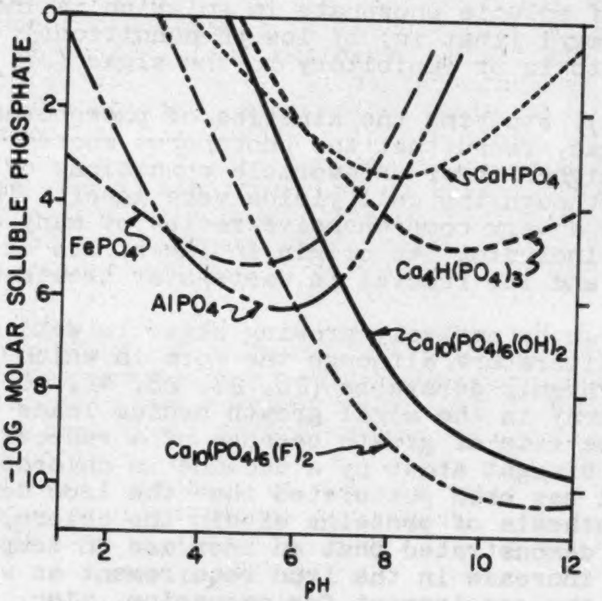

FIGURE 32- PHOSPHATE SOLUBILITY AS A FUNCTION OF pH

FROM STUMM AND LECKIE (22)

One of the most important reactions plotted in Figure 32 is the exergonic interaction in the formation of hydroxyapatite:

$$10\ CaCO_3(s) + 6HPO_4^{-2} + H_2O + 2H^+ = Ca_{10}(PO_4)_6(OH)_2 + 10\ HCO_3^- \qquad \text{Eq. [8]}$$

It is thought to be one of the principal control mechanisms for the exchange of phosphates between the sediments and overlying waters. Precipitates of metals, which are formed at the pH normally encountered in algal systems, can also cause some phosphate removal from the medium, either by precipitation as an insoluble salt or by adsorption upon some insoluble substance (21, 22, 26, 43, 61, 63, 64). The extent of phosphorus precipitation is increased under aerobic conditions and conversely is decreased under anaerobic conditions, as a result of oxidation-reduction changes. Bongers (24) and others also found that algae settling rates vary according to the coagulation effect of the insoluble phosphate salts produced at high pH levels. This finding responds to that by Steele and Yentsch (28), who noted that as cells aged or nutrients became depleted, the rate of algal settling increased. Conversely, if for any reason the concentration of soluble phosphate in solution is increased beyond 5 to 20 mg/l (that is, by low pH conditions), phosphorus becomes toxic or inhibitory to the algae (27, 42).

Zabat et al (65), studying the kinetics of phosphorus assimilation by algae, found that the phosphorus content per unit cell mass was higher under unfavorable conditions of pH and temperature, although the cell yields were lower. Their report presents a very comprehensive review of many aspects of phosphorus, including its origin in lakes, its assimilation by algae, and its removal in wastewater treatment plants.

The need for iron by actively growing algae is well substantiated in the literature, although the form in which it can be utilized is highly debatable (26, 27, 28, 42, 66, 67). An iron deficiency in the algal growth medium leads to a reduction in the rate of growth because of a reduction of photosynthesis brought about by a decline in chlorophyll production. It has been postulated that the iron deficiency reduces the synthesis of proteins within the chloroplast. It was further demonstrated that an increase in temperature caused a sharp increase in the iron requirement as well as an increase in the requirement for magnesium, zinc, and manganese (28).

Excess iron can be toxic, depending upon algal species (27, 28). According to Provasoli and Pinter (42), a given concentration of iron can be in excess (toxic) at one pH level and be deficient at a slightly different pH. The change in iron solubility with pH and oxidizing-reducing conditions has been well documented. Morgan and Stumm (68) have made a comprehensive review of the chemistry of iron and manganese in limnological cycles. In Figures 33 and 34, taken from their paper on "The Role of Multivalent Metal Oxides in Limnological Transformations, as Exemplified by Iron and Manganese", the changes in iron and phosphorus solubility are shown as a function of pH. As indicated

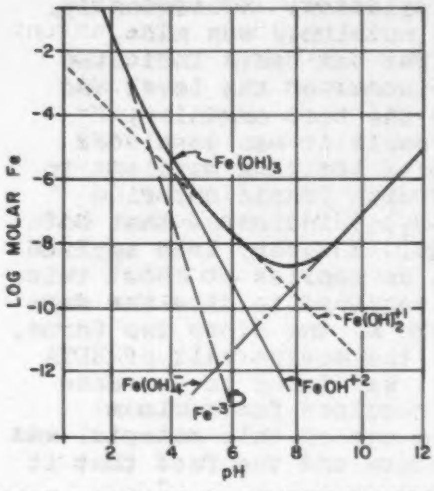

FIGURE 33 - SOLUBILITY OF FERRIC HYDROXIDE IN WATER AT 25°c
FROM MORGAN AND STUMM(68)

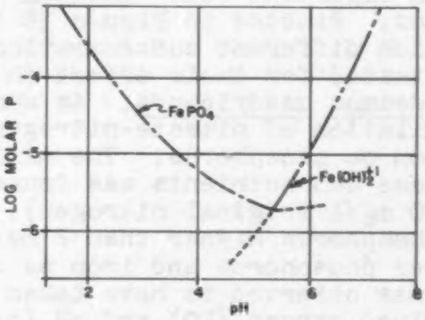

FIGURE 34 SOLUBILITY OF FERRIC PHOSPHATE IN WATER AT 25°c
FROM MORGAN AND STUMM(68)

by the curves in Figures 33 and 34, very little iron is soluble at the pH levels expected in systems with even minimal algal growth. According to Hutchinson (67), ferric iron can be present in excess of 0.01 ppm only as a suspension of oxides or hydroxides in aerated waters in which the pH is above 5. Phosphate solubility in lakes has also been correlated to that of iron. Both of these important algal nutrients are known to be released from the sediments under anaerobic conditions and low pH levels.

The nitrogen-to-phosphorus ratio in the IAWTC tile drainage was about 100 to 1 (based on a 20 mg/l nitrate-nitrogen influent), as compared to about 10 to 1 in the algal cell. As a result, phosphorus was the first nutrient studied in Phase I, and was found to be insufficient in the tile drainage for maximum nitrogen assimilation. Subsequently, a general screening of known algal nutrients was made, utilizing IAWTC tile drainage. Light box tests indicated that the addition of iron greatly increased the level and extent of nitrogen assimilation by the test organism, *Scenedesmus quadricauda*. On this basis it was concluded that iron as well as phosphorus was a limiting nutrient in the IAWTC water. Additional tests with ferric chloride ($FeCl_3$) and ferric sulfate ($Fe_2(SO_4)_3$) indicated that both forms were adequate sources of iron. However, iron applied as ferric citrate (FeC_6H_5O) had to be applied at about twice the concentration (as Fe) of that required to give the same effect on growth and nitrogen uptake as the other two forms. The addition of a chelating agent, the sodium salt of EDTA (ethylenediamine tetraacetic acid), was found to decrease the optimum concentration of iron required for maximum growth and nitrogen uptake, but the use of this material was discontinued because of its high costs and the fact that it contributes nitrogen to the system.

The beneficial effect of iron and phosphorus on nitrogen assimilation is reiterated in this report because a reevaluation of data indicated that iron in addition to phosphorus was an important rate-limiting nutrient in many of the IAWTC studies. Plotted in Figure 35 are data obtained in one study in which different concentrations of iron and phosphorus were tested for their effect on nitrogen assimilation by *Scenedesmus quadricauda*. As shown in this figure, little assimilation of nitrate-nitrogen took place in the absence of iron or phosphorus. The minimal effective level of each of these two nutrients was found to be approximately 2 mg/l (at 20 mg/l original nitrogen). At concentrations of iron and phosphorus higher than 2 mg/l, a definite interaction between phosphorus and iron as related to nitrogen assimilation was observed to have taken place. Since high levels of dissolved oxygen (DO) and pH (over 9) are associated with high rates of nitrate assimilation during photosynthesis,

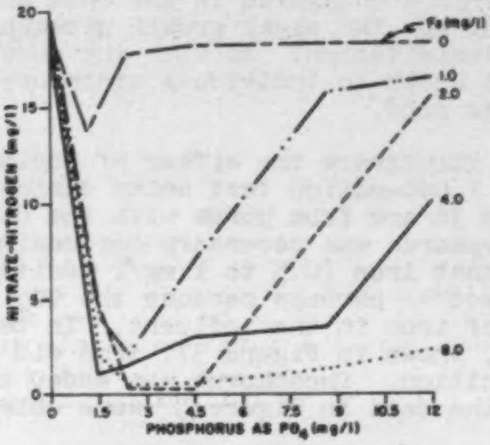

FIGURE 35-EFFECT OF VARYING IRON AND PHOSPHORUS CONCENTRATIONS ON NITROGEN ASSIMILATION-LIGHTBOX STUDY

undoubtedly this interaction corresponded to the coprecipitation of iron and phosphorus under active growth conditions. The maximum extent of nitrogen assimilation in the laboratory cultures occurred when 2 to 3 mg/l of both iron and phosphorus were added.

In the tests on the effect of adding iron, the units not receiving carbon dioxide were operated from April through September 18, 1970. After the latter date, the unit not receiving iron was used in a temperature study. In this unit, the addition of iron consistently improved the total nitrogen assimilation by approximately 3 to 5 mg/l. Apparently, carbon dioxide addition not only made up for the carbon deficiency but, by lowering the pH level, also increased the solubility of iron and possibly other nutrients in the tile drainage.

In the Phase I investigation, iron had been added to the test units at concentrations ranging from 3.0 to 6.0 mg/l $FeCl_3$ as Fe. During the four to six weeks duration of these studies, upwards of 30 mg/l iron were found to accumulate in the test units in a nonfilterable form. Iron in solution was usually at concentrations less than 0.1 mg/l, the lower limit of detection with the method used.

Because nonsoluble iron has been reported to be assimilable by algae, the iron concentration in the Phase II investigation was decreased from 3.0 mg/l to 1.0 mg/l or less on the assumptions that iron accumulated in the unit and that it would be sufficient for the algal growth necessary for maximum nitrogen assimilation. As with the phosphorus addition, iron was added to individual miniponds rather than to the storage pond.

Figures 36 and 37 illustrate the effect of adding iron and phosphorus to the 1,000-gallon test units during 1970. The data in Figure 36 are from ponds with the CO_2 addition and show that phosphorus was necessary for maximum nitrogen assimilation but that iron (0.5 to 1 mg/l addition) had no beneficial effect -- perhaps because the CO_2 increased the availability of iron in the influent. In those ponds not receiving CO_2, shown in Figure 37, iron did appear to be a necessary addition. Phosphorus was added to both units from which the data in Figure 37 were obtained.

The data plotted in Figure 38 show the changes in the influent and the average effluent orthophosphate concentrations characteristic of all of the miniponds receiving phosphorus. They also indicate phosphate concentration of the effluent from the unit which did not receive phosphate. From these data, it was concluded that 80 to 90 percent of the influent phosphate either was assimilated by the algae or was precipitated out of solution. In addition, the figure shows the average phosphate concentration in the cultures in all of the test units as well as the influent nitrogen and phosphate concentration. Apparently, as the concentration of influent nitrogen decreased, excess phosphate, that is, that not assimilated or precipitated, went into solution.

During the month of July, most miniponds were emptied, repaired, cleaned, and restarted with a common inoculum from the 1/4-acre demonstration unit. Several months later, samples of sludge from all the units were analyzed for organic and inorganic constituents. The results of these analyses are plotted in Figure 39 and listed in Table 3. They indicate that, even during mixing, up to 99 percent of the total solids in the units were present as a sludge on the bottom of the unit in the form of: (1) carbonates, (2) phosphates, (3) iron, and (4) algal material. Further calculations indicated that much of the iron, phosphate, and carbon added during the two-to-three-month period could be accounted for in the sludge. This precipitation occurred to a larger extent than in the Phase I studies and was probably accumulative throughout the period.

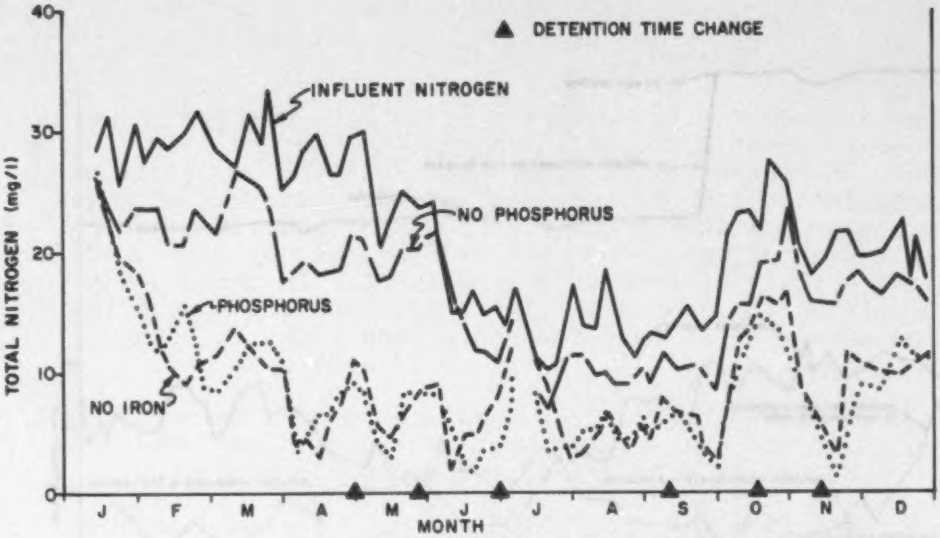

FIGURE 36-EFFECT OF IRON OR PHOSPHORUS ADDITION ON NITROGEN ASSIMILATION WITH CARBON DIOXIDE ADDITION

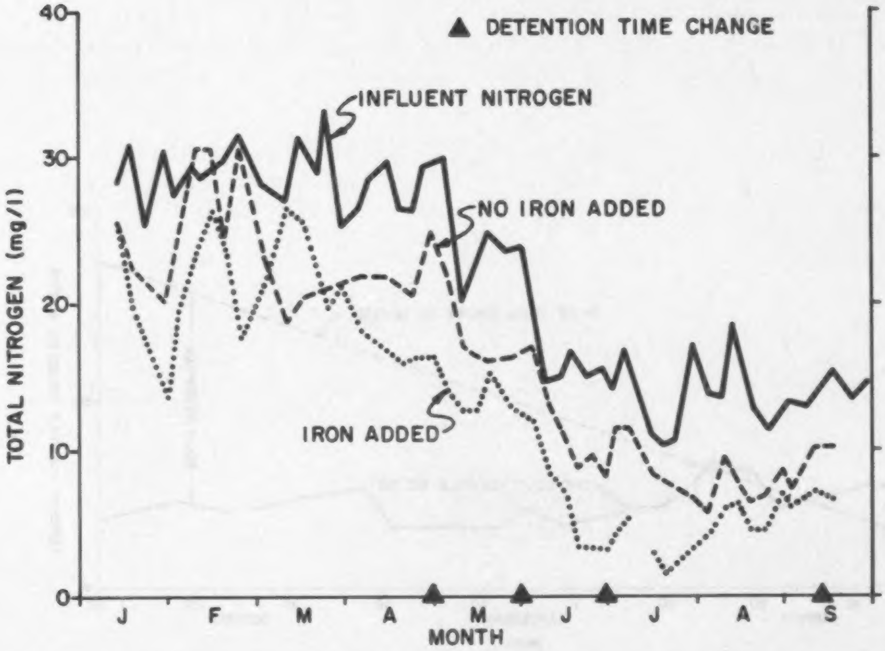

FIGURE 37-EFFECT OF IRON ADDITION ON NITROGEN ASSIMILATION WITHOUT CARBON DIOXIDE ADDITION

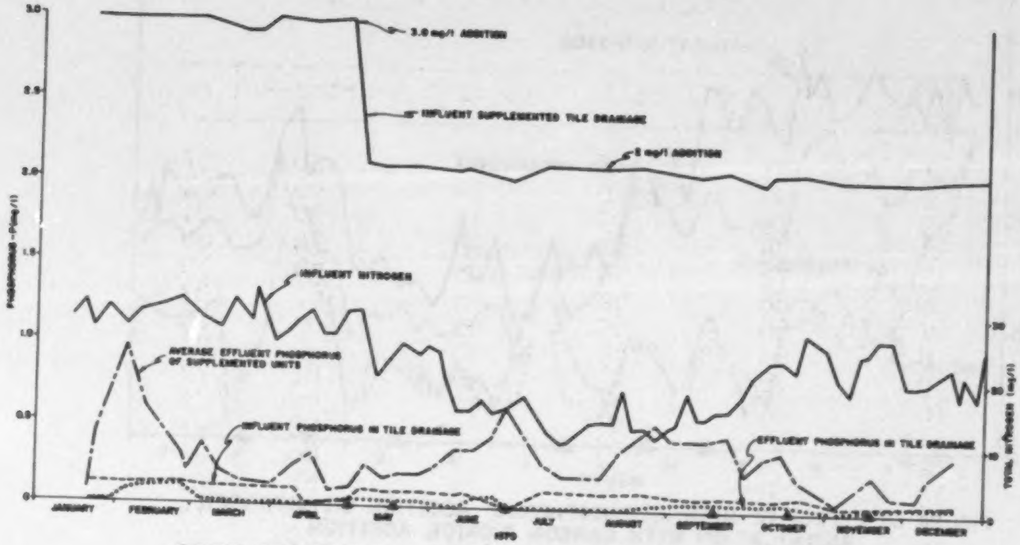

FIGURE 38-INFLUENT AND EFFLUENT PHOSPHORUS CONCENTRATIONS IN UNITS WITH NORMAL AND PHOSPHORUS SUPPLEMENTED TILE DRAINAGE

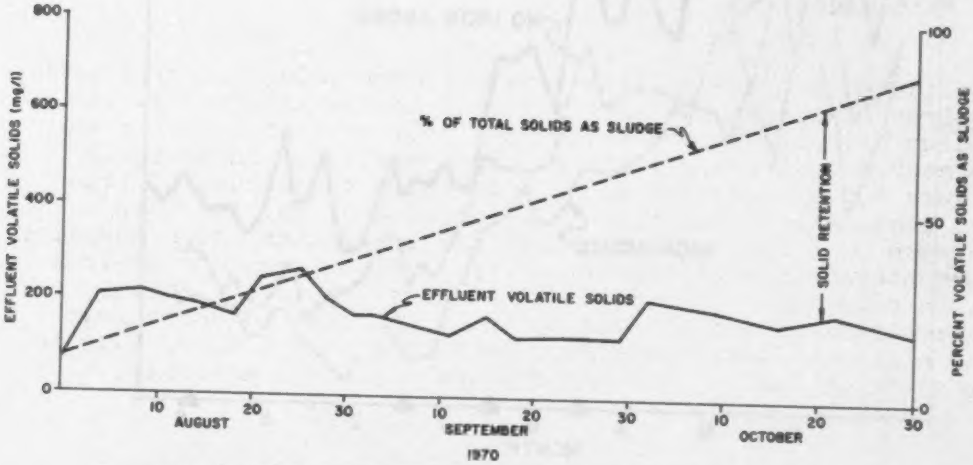

FIGURE 39-TYPICAL SLUDGE ACCUMULATION IN TEST UNITS

TABLE 3

PERCENT OF TOTAL POND MATERIALS FOUND IN POND SLUDGE--DURING MIXING

Unit No.	Filterable Solids	$CaCO_3$	Total P	Ortho-P	Fe
1	87	8	47	38	57
2	93	48	94	85	82
3	79	3	--	30	91
4	74	61	95	75	66
5	72	59	95	66	70
6	86	62	84	61	83
7	86	69	99	93	86
8	85	45	100	--	71
9	76	32	95	81	77
10	81	32	100	100	72
11*	99	83	85	68	--
12*	99	77	58	49	--
13	89	97	98	87	65
14	87	26	100	100	74
15	74	66	100	100	68
16	88	74	100	100	33
17*	92	49	100	100	85
18	91	88	48	92	82
19	92	86	100	82	80
20	98	91	100	99	90
21*	99	80	71	27	--
22*	99	72	58	50	--

*Not mixed.

Replicated samples of tile drainage containing 2.0 mg/l each of iron and phosphate were adjusted in the laboratory with sodium hydroxide to raise the pH level to that normally encountered in the algal units. The results of these tests, Figure 40, showed that in tile drainage the amount of iron and phosphate in solution is a function of pH, and that between pH 8 and 9, levels that are lower than those normally encountered in algal systems, very little phosphate or iron is in solution. Therefore, it appears that: the actual concentrations assimilated by the algae must be quite low; the algae can assimilate some nutrients in the dark, that is, when the pH is low; or they can use colloidal material.

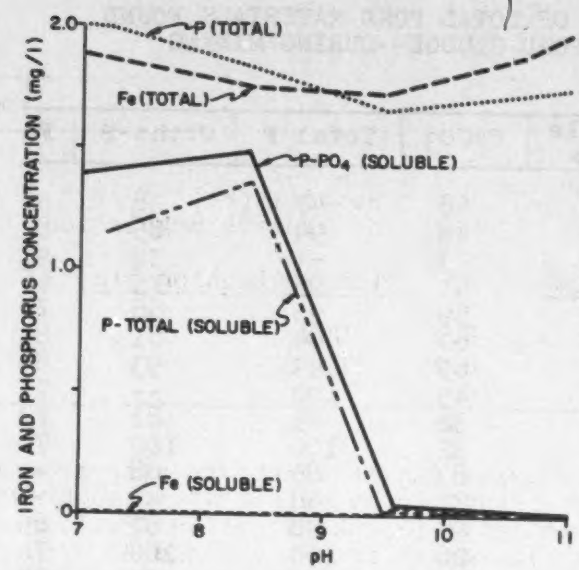

FIGURE 40-IRON AND PHOSPHORUS SOLUBILITY AT DIFFERENT pH VALUES

In Table 4 are listed the average phosphorus changes in the effluent from the various units during 1970. The data indicate that: (1) mixing tends to precipitate phosphate, (2) the continual removal of sludge decreases the soluble phosphate, and (3) phosphate loss is related to unit loading and carbon availability. These conclusions agree with the changes predicted for phosphate and iron solubility as shown in Figures 32, 33, and 34, specifically that, at high pH levels and with the culture in an aerobic condition, phosphate and iron will be in an insoluble form. The data led to the conclusion that the actual availability of these nutrients in the nonsoluble form was quite limited, and, hence, the requirements of these nutrients must be quite low because the nitrogen assimilation did not appear to be adversely affected.

In the last few months of Phase II, iron was added directly to the biomass control settling tanks of the 1/4-acre demonstration unit and to several miniponds, although phosphate was still added to the demonstration unit directly. This was done to decrease the precipitation of phosphorus and iron. Analysis of data from the units indicated that: (1) iron was being picked up by the algae as they passed through the

TABLE 4

AVERAGE DISSOLVED EFFLUENT PHOSPHATE IN MINIPOND DURING 1970

Unit	mg/l-P	Unit	mg/l-P
Mixed	0.2	Nonmixed	0.4
Soil (no Fe)	0.6	Nonsoil (Fe)	0.3
Biomass Regulation	0.2	No Biomass Regulation	0.5
Short Detention Time		Long Detention Time	
CO_2	0.5	CO_2	0.2
No CO_2	0.25	No CO_2	0.1

biomass regulation device (any attempts to stop iron addition during Phase II were found to be detrimental, apparently because iron was essential); (2) the volatile fraction of the effluent solids was increased from the range 40 - 50 percent to 60 - 70 percent; and (3) the ability of the algae to remain in suspension was enhanced. An effect of directly adding iron in this manner will be discussed in detail in the chapter, "Algal Harvesting and Disposal".

It is postulated that the accumulation of inorganic and organic material, as indicated by the data in Table 3 and the curves in Figure 39, may be detrimental to maximum nitrogen assimilation in that: (1) nutrients become unavailable to the algae because of precipitation, (2) the accumulation of inorganic compounds flocculates much of the viable photosynthesizing algae, (3) the suspended solids present greatly interfere with light availability to the algae in the mixed systems, (4) the re-solubilization of the nutrients may bring about the production or release of toxic substances, and (5) decomposition of the accumulated sludge may release nitrogen which can add to the total soluble nitrogen in the system.

From these and similar studies it was concluded that: (1) iron and phosphate are essential to maximum nitrogen assimilation in IAWTC tile drainage, (2) the method of nutrient addition is very important, and (3) because of the factors listed above, the type of algal reactor tested at the IAWTC (with mixing) would probably not permit optimal algal growth and maximum nitrogen assimilation on a long-term basis.

Effect of Sludge Accumulation and Decomposition on Nitrogen Assimilation

Although not normally considered a growth regulatory factor, sludge accumulation (algae, detritus, and inorganic nutrient precipitates) can affect the nitrogen removal efficiency of algal wastewater treatment systems in a number of ways. According to Foree (29), the nature of the factors on which the coefficients a, b, c, d and e in Equation [1] are based determines the chemical composition of the algal matter synthesized, and varies according to the species and age of the algae, temperature, available nutrients, and other related factors. Jewell and McCarty (20) and Foree and McCarty (30), studying both the aerobic and anaerobic decomposition of algae, found that a large fraction of the initial particulate nitrogen and phosphorus was not regenerated (40 to 60 percent) but remained in the undecomposed (refractory) particulate material after active decomposition and regeneration appeared complete. Conversely, Golterman (26) reported that nitrogen is rapidly re-mineralized after the death of algae and, in the shallow waters of lakes, algae would probably be broken down before they reached the mud.

Regardless of the extent of decomposition, the nutrients released from algal decomposition have been found to be an excellent source of nitrogen and other nutrients for algal growth (31). As shown by the diagram in Figure 1, the only nitrogen form directly resulting from the decomposition of algal sludge is ammonia (21, 64). Ammonia, in turn, is readily utilized by the actively growing algae in preference to other forms of inorganic nitrogen, such as nitrate or nitrite (19, 23, 28, 34, 35). In the course of time, under aerobic conditions, ammonia is oxidized back to nitrate by nitrifying bacteria. Hence, through algal accumulation and decomposition, a variable amount of nitrogen will be recycled in the system. Most likely, the extent of the sludge nutrient recycle (decomposition) is a function of the degree of sludge accumulation and temperature (30).

A test performed in January 1971, in which 16 mg/l of ammonia was added to a minipond unit which had been removing a fairly constant level of nitrogen, indicated that ammonia was assimilated preferentially to nitrate. The data plotted in Figure 41 show that the ammonia concentration in the effluent decreased faster than was predicted by dilution alone. The observed difference was assumed to be caused by the preferential uptake of the ammonia-nitrogen by the algae, although nitrification could have been partially responsible. It is postulated that if this fact were not recognized, for example, during periods of elevated decomposition, the assumption could be made that the algae were assimilating less nitrogen than the unit influent and effluent level would indicate,

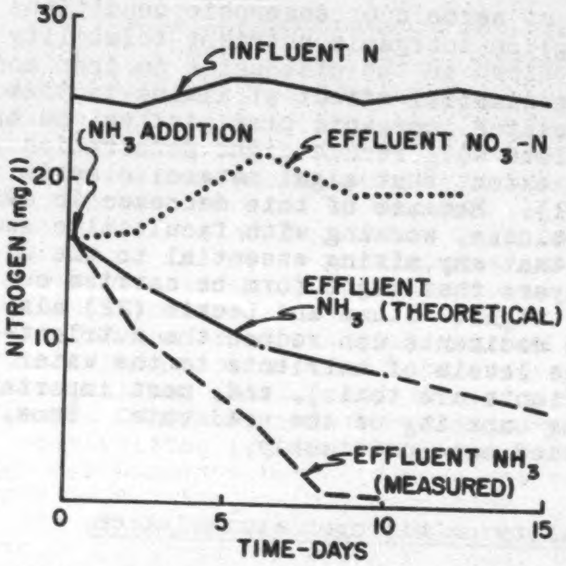

FIGURE 41-PREFERENTIAL ALGAL UPTAKE OF AMMONIA OVER NITRATE

while in actuality they might have been utilizing more than the influent level. Accordingly, it would seem that if the system were not mixed (anaerobic), denitrification rather than nitrification would take place, which might benefit total nitrogen removal. Although this test was performed only one time during the winter at low growth rate levels, what happened in the test appears to be a reasonable explanation of what probably occurred during certain times of the year in the test units.

Assimilation of ammonia by algae in preference to nitrate is the result of the fact that a lesser amount of energy is required for assimilating ammonia, and that ammonia can repress both the nitrate uptake mechanism and nitrate reductase formation in the nitrogen-reducing reaction (34). Furthermore, the rate of ammonia assimilation is much more rapid than that of nitrate assimilation or that of the subsequent conversion of nitrogen to protein (35). Thus, the accumulation and subsequent decomposition of algal sludge can affect nitrate uptake.

Another beneficial effect of algal sludge decomposition reported by Provasoli and Pinter (42) to take place in nonmixed algal systems is the release of organic acids (especially amino acids) which can act as trace-element chelators; however, no attempt was made to determine whether this phenomenon had any effect on the IAWTC system.

The various effects of aerobic or anaerobic conditions (mixing or nonmixing) on inorganic nutrient solubility in the sludge were described in the discussion on iron and phosphate. A further detrimental effect of mixing is that the suspension of accumulated inorganic precipitates and of nonphotosynthesizing algae will reduce light penetration into the culture, to the extent that algal metabolic activity is greatly retarded (31). Because of this decrease in available light, Oswald and Golueke, working with facultative sewage ponds, recommended that any mixing essential to the disruption of any anaerobic layers that might form be carried out only for 2 to 4 hours at night. Stumm and Leckie (22) also found that disturbing the sediments can reduce the nutrient-binding effect, expose large levels of nutrients to the water (excesses of some nutrients are toxic), and, most importantly, reduce the buffering capacity of the sediments. Thus, mixing must be carried out judiciously.

Effect of Water Quality on Nitrogen Assimilation

The trace elements (micronutrients) considered necessary for sustained algal growth were listed in a previous section. Their solubility as well as availability to algae are basically similar to those described previously for iron and phosphorus. The amount of a specific trace element required varies greatly according to algal species and level of growth (28). Summarized in Table 5 are the micronutrients normally required for certain specific algal metabolic activities (43).

TABLE 5

SUMMARY OF MICRONUTRIENT
TRACE MINERAL REQUIREMENTS*

Process	Trace Element Required
Photosynthesis	Manganese, iron, chloride, zinc, vanadium
Nitrogen Fixation	Iron, boron, molybdenum, cobalt
Other Functions	Manganese, boron, cobalt, copper, silicon

*Table from Fruh (43).

As with all algal nutrients, the total concentration of a particular micronutrient in the growth medium is practically meaningless, unless it is in a form capable of being assimilated by the algae.

During the course of the Phase I and II investigations, several micronutrients were evaluated for their potential effect on nitrate assimilation. These studies were usually only conducted once, with water of a specific quality. As indicated in Figure 3, the quality of the water changed greatly throughout the year, and, as a result, a variation in response would be expected at different times of the year.

The micronutrients tested in the light box (at low concentrations) were molybdenum (Mo), vanadium (V), manganese (Mn), zinc (Zn), and potassium (K). Of these micronutrients, manganese and potassium were found to improve assimilation of nitrate by Scenedesmus quadricauda under the test conditions, depending upon the level of carbon available (that is, function of pH level). When K and Mn were added to several miniponds, no measurable response in nitrogen uptake was noted. However, these tests were not definitive because, in conducting them, consideration was not given to the general constituents of the water or to the specific growth rate of the algae which can affect the nutrient requirements. In the light box studies, there was little response in improved nitrogen uptake with Mo, V, or Zn addition; however, Zn was found to appreciably increase the ability of the algae to remain in suspension. Although these tests were very limited and inconclusive, it is suggested that in any future work a continuous nutrient check of the plant influent be made to maintain a growth medium that will support extensive algal growth and nitrogen assimilation.

During the course of this investigation, several light box tests were conducted to determine if there would be any differences in biological response to water from different tile drainage systems in the San Joaquin Valley. In each of these studies, the nitrogen, phosphorus, carbon, and iron concentrations were brought to identical levels, and the algae were cultured under similar light and temperature conditions at the same low level (2,000 cells per ml) of algal inoculum, Scenedesmus quadricauda. In other words, all major growth factors were presumably equal. Therefore, any difference in nitrogen assimilation was assumed to be the result of some factor or factors other than those named in this paragraph.

In the tests, the rate and amount of algal nitrogen assimilation were usually noted to vary significantly according to the tile drainage used in the medium. For example, in the last run of the series, tile drainage was collected from 12 different areas in the San Joaquin Valley. Special care was taken to assure that the tile drainage came from fields with different agricultural crops and varying soil composition. The tile drainages were then spiked with nitrate-nitrogen to 50 mg/l, the level of the highest drainage collected, and varying concentrations of phosphorus, iron, and carbon were added to the flasks. Comparison of nitrogen assimilation by *Scenedesmus quadricauda* over a three-week period showed that there was a significant variation between the different tile drainages. Algae that were cultured in sump B tile drainage (see Figure 42) assimilated 50 mg/l NO_3-N in about 10 days, while at the other extreme algae grown in sump A tile drainage had only assimilated 25 mg/l NO_3-N in 25 days. The IAWTC tile drainage, sump I, along with the other nine tile drainages (which are not included in Figure 42), were intermediate in level and time necessary for complete nitrate assimilation.

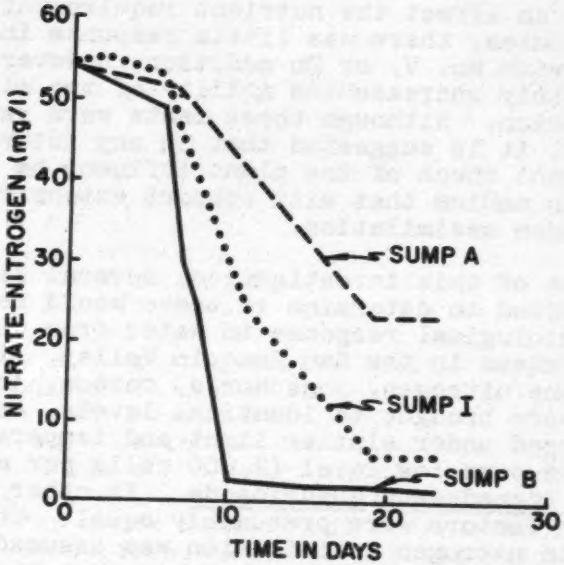

FIGURE 42- NITROGEN ASSIMILATION IN TILE DRAINAGE FROM THREE SUMPS

A comparison of the water quality constituents (TDS, alkalinity, trace elements, etc.) indicated that the only apparent major difference between tile drainage in which nitrogen removal was greatest and that in which it was least was the level of molybdenum. The concentration of molybdenum in the former was 34 parts per trillion (ppt) and that in the latter was 9 ppt. However, in view of the fact that some precipitation of nutrients may have taken place, these values do not necessarily represent the concentration of molybdenum actually available to the algae.

On the basis of this study, it was concluded that the combined San Joaquin Valley drainage would largely buffer the effect of changes in water quality often noted at the IAWTC, and that the chance of any minor nutrients limiting growth and nitrogen assimilation would be slight.

To determine the effect of water quality changes on nitrogen assimilation during Phases I and II, filtered samples of the influent and growth unit effluent were analyzed for trace elements each time the storage pond was filled during most of 1970. The results of the analysis of the concentrations of trace elements in filtered and unfiltered samples are listed in Table 6.

Of the 17 trace elements monitored in 1970, Al, Cd, Cr, Co, Cu, Fe, Pb, Mn, Mo, Ni, V, and Zn fluctuated during the year. The concentrations of Be, Bi, Ga, Ge, and Ti remained below analytical detection levels. However, changes in algal response after filling of the storage pond could not be related to the fluctuations. Figure 43 illustrates the effect of filtering (0.45μ membrane filters) on the concentration of six trace elements in influent and 1/4-acre demonstration unit water. These samples were collected and filtered on October 27, 1970. The bar graphs show that in most instances there were higher concentrations of the elements in the pond than in the influent, perhaps because of concentration by the algal cells. Except for aluminum in the influent and molybdenum in both influent and pond samples, there was little difference between the concentration in the filtered and unfiltered samples. The lack of difference is rather surprising because the unfiltered samples included algal cells and precipitates and presumably more of the trace minerals. The form in which many of the trace nutrients are available to algal cells, either dissolved or particulate, is not completely known, thus, future studies should consider running analyses on both the total sample and the filtrate.

TABLE 6

TRACE ANALYSIS - 1970
(In micrograms/liter)

Sample	Date	Al	Be	Bi	Cd	Cr	Co	Cu	Ga	Ge	Fe	Pb	Mn	Mo	Ni	Ti	V	Zn
Influent (filtered)	2/13	L1.4	L0.6	L0.3	29	L1.4	L1.4	31	L5.7	L0.3	7.1	L1.4	5.7	54	7.7	L0.6	2.2	L5.7
	3/17	11	L0.6	L0.3	18	2.3	L1.4	34	L5.7	L0.3	2.6	L1.4	11	51	6.0	L0.6	19	29
	5/21	L1.4	L0.6	L0.3	L1.4	L1.4	L1.4	L1.4	L5.7	L0.3	19	L1.4	L1.4	71	70.3	L0.6	1.9	L5.7
	7/15	60	L0.6	L0.3	19	28	L1.4	L1.4	L5.7	L0.3	10	L1.4	7.1	14	4.9	L0.6	1.4	L5.7
	7/27	63	L0.6	L0.3	L1.4	L1.4	629	L1.4	L5.7	L0.3	G100	L1.4	13	37	46	L0.6	3.4	L5.7
	8/12	20	L0.6	L0.3	L1.4	L1.4	20	L1.4	L5.7	L0.3	20	L1.4	4.6	11	26	L0.6	2.1	L5.7
	8/25	L1.4	L0.6	L0.3	L1.4	L1.4	L1.4	L1.4	L5.7	L0.3	8.9	9.1	7.4	3.4	8.0	L0.6	1.7	L5.7
	.10/27	G1.4	L0.6	L0.3	26	31	L1.4	L1.4	L5.7	L0.3	2.9	L1.4	14	23	9.1	L0.6	3.4	L5.7
	11/20	27	L0.6	L0.3	37	51	L1.4	L1.4	L5.7	L0.3	7.1	L1.4	6	54	9.7	L0.6	2.3	L5.7
(unfiltered)	10/27	46	L0.6	L0.3	31	31	L1.4	L1.4	L5.7	L0.3	4.9	L1.4	12	140	8.0	L0.6	2.0	L5.7
Growth Unit (filtered) No. 1	2/13	L1.4	L0.6	L0.3	13	L1.4	L1.4	15	L5.7	L0.3	9.4	L1.4	3.4	40	4.0	L0.6	2.5	L5.7
No. 17	3/17	L2.5	L1.0	L0.5	7	L2.5	L2.5	L2.5	L10	L0.5	3.2	L2.5	L2.5	25	L0.5	L0.5	0.8	50
¼ acre	5/21	L1.4	L0.6	L0.3	L1.4	L1.4	L1.4	57	L5.7	L0.3	26	L1.4	L1.4	54	L0.3	L0.6	1.1	L5.7
No. 17	6/2	L1.4	L0.6	L0.3	L1.4	L1.4	L1.4	L1.4	L5.7	L0.3	31	L1.4	L1.4	37	4.3	L0.6	1.4	L5.7
No. 6	7/21	23	L0.6	L0.3	19	31	L1.4	L1.4	L5.7	L0.3	9.1	29	L1.4	24	4.3	L0.6	2.5	L5.7
No. 6	8/21	21	L0.6	L0.3	L1.4	L1.4	L1.4	L1.4	L5.7	L0.3	7.4	L1.4	L1.4	20	4.0	L0.6	2.3	L5.7
No. 7	11/20	L1.4	L0.6	L0.3	49	4.3	L1.4	L1.4	L5.7	L0.3	4.6	L1.4	L1.4	290	7.1	L0.6	1.0	230
¼ acre	10/27	220	L0.6	L0.3	54	57	L1.4	L1.4	L5.7	L0.3	G60	L1.4	8.9	37	11	L0.6	2.3	L5.7
(unfiltered)	10/27	180	L0.6	L0.3	54	57	L1.4	L1.4	L5.7	L0.3	G60	L1.4	L1.4	89	13	L0.6	1.8	L5.7
Influent* (unfiltered minus filtered)	10/27	45	0	0	5	0	0	0	0	0	2.0	0	0	117	0	0	.3	0
Growth Unit* (unfiltered minus filtered)	10/27	193	0	0	40.9	55	0	0	0	0	47	0	3.6	23	18.5	0	.3	0

*Difference due to the presence of insoluble form and/or concentrated in the algae.

G - Greater than.

L - Less than.

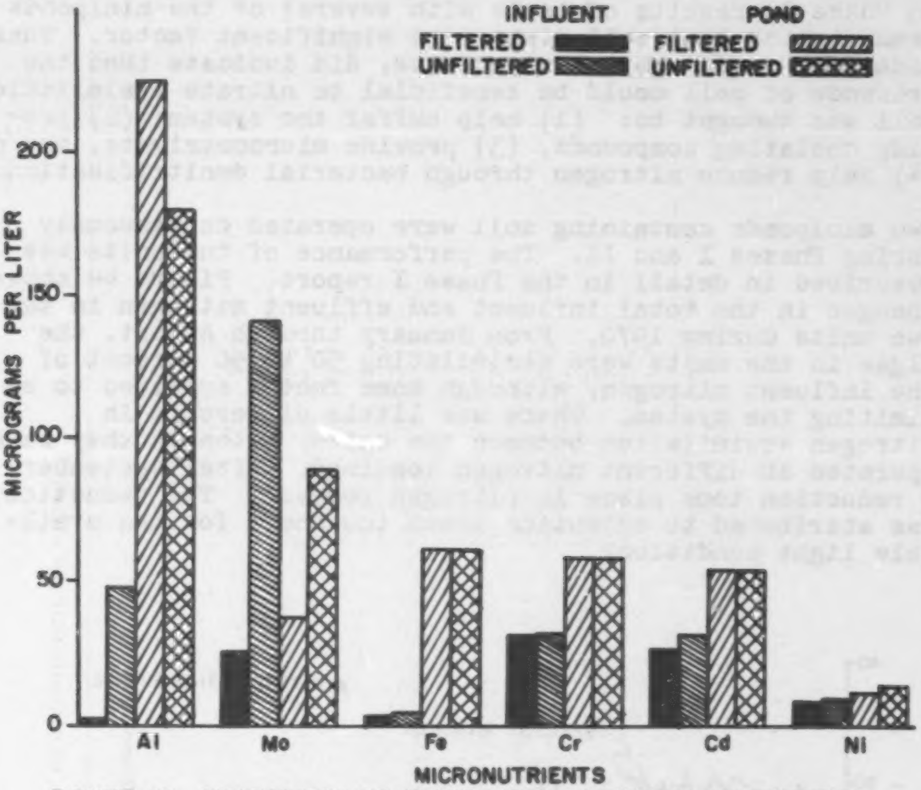

FIGURE 43 COMPARISON OF TRACE METAL ANALYSIS OF FILTERED AND UNFILTERED TILE DRAINAGE

Another aspect of the relation between change in water quality and nitrate assimilation was the accumulation of TDS, resulting from evaporation from the test units. An examination of the yearly average percent increase in the TDS in the growth units shows that accumulation of TDS was a function of flow and evaporation. There was a minimum of 8 percent increase in TDS and a maximum of 31 percent (shallow units with long detention times). Average increases appear to have ranged between 15 and 20 percent. There was a net TDS accumulation in all units, but there was no obvious effect on algal growth response.

Effect of Soil on Nitrogen Assimilation

Several other studies considered pertinent to the Phase II investigation were carried out by means of flask bioassays. They were designed to determine whether or not the presence or absence of soil had any influence on nitrate assimilation.

In Phase I, results of tests with several of the miniponds demonstrated that soil might be a significant factor. These bioassays, although not conclusive, did indicate that the presence of soil could be beneficial to nitrate assimilation. Soil was thought to: (1) help buffer the system, (2) provide chelating compounds, (3) provide micronutrients, and/or (4) help reduce nitrogen through bacterial denitrification.

Two miniponds containing soil were operated continuously during Phases I and II. The performance of the units was described in detail in the Phase I report. Figure 44 shows changes in the total influent and effluent nitrogen in the two units during 1970. From January through August, the algae in the units were assimilating 50 to 90 percent of the influent nitrogen, although some factor appeared to be limiting the system. There was little difference in nitrogen assimilation between the units, although they were operated at different nitrogen loadings. After September, a reduction took place in nitrogen removal. The reduction was attributed to detention times too short for the available light conditions.

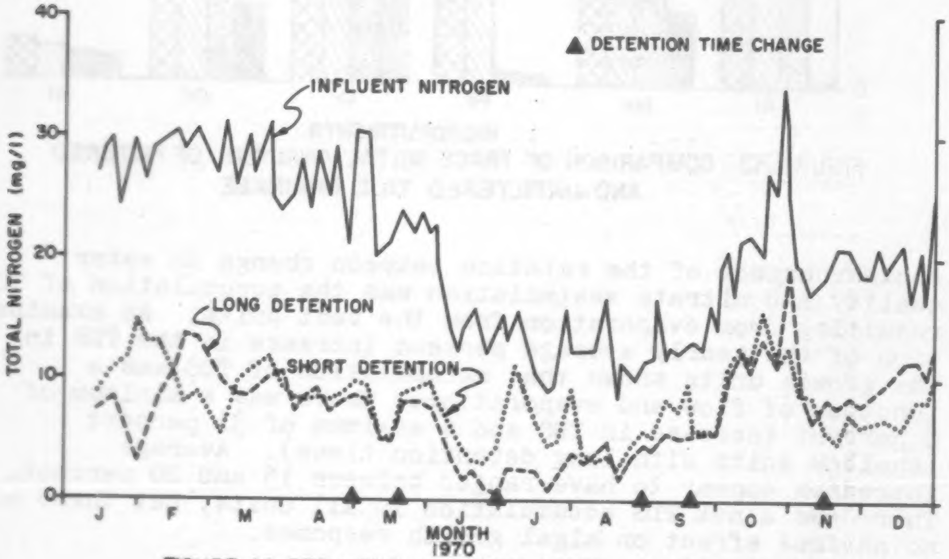

FIGURE 44-EFFLUENT NITROGEN LEVEL IN SOIL UNITS

A comparison of the performance of the units containing soil with that of units that were not mixed and contained no soil indicated that there was essentially no difference in performance between the two systems. Consequently, it was concluded that the accumulation of organic material, and not soil specifically, was responsible for the maintenance of nitrogen assimilation efficiencies in the units. As will be shown later, the algae grown in the two sets of units contained twice the amount of nitrogen found in the cells of algae cultured in the units that were mixed.

Special studies were conducted in the summer and in the fall of 1970. Four 10-inch diameter glass cylinders (two opaque and two clear) were placed in a growth unit containing soil which had been enriched with nitrate, phosphate, and iron. The soil was removed from one opaque and one clear cylinder, and the nitrate-nitrogen level was adjusted to 20 milligrams per liter in each cylinder. The result of the summer and fall studies are shown in Tables 7a and 7b.

TABLE 7a

NITRATE IN THE WATER OF OPAQUE AND CLEAR CYLINDERS (SUMMER TEST)

(expressed as nitrogen, in mg/l)

Date	Opaque Cylinders				Clear Cylinders			
	Without Soil		With Soil		Without Soil		With Soil	
	NO_3-N Level	NO_3-N Added	NO_3-N Level	NO_3-N Added	NO_3-N Level	NO_3-N Added	NO_3-N Level	NO_3-N Added
7/31	20		20		20		20	
8/04	9.2		5.6		6.2		0	
8/05	7.8		3.0		5.6	+20	0	+20
8/05	-		-		20		24.8	
8/07	6.7		0.7	+20	17		1.8	+20
8/10	7.0		16.4		13		1.7	+20
8/10	-		-		-		21.8	
8/12	6.0		39.7		6.8		12.2	
8/13	5.5		58.8		9.8		38.9	
8/14	6.8		54		8.6		30	
8/15	6.1		45		7.6		20.2	
8/17	5.7		42		3.6		7.4	
8/18	4.8		40		2.1		2.7	
8/19	3.6		32		1.2		1.4	
8/21	4.3		30		0.8		0.8	

TABLE 7b

NITRATE IN THE WATER OF OPAQUE AND CLEAR CYLINDERS (FALL TEST)

(expressed as nitrogen, in mg/l)

Date	Opaque Cylinders				Clear Cylinders			
	Without Soil		With Soil		Without Soil		With Soil	
	NO_3-N Level	NO_3-N Added	NO_3-N Level	NO_3-N Added	NO_3-N Level	NO_3-N Added	NO_3-N Level	NO_3-N Added
9/16	20.2		14.0		19.6		4.4	
9/17	19.6		11.0		19.4		4.3	
9/18	22.6		8.8		21.4		4.0	+20
9/22	20.2		1.2	+20	16.5		3.2	
9/23	20.2		19.0		15.3		3.2	
9/24	15.3		19.2		12.8		4.3	
9/25	17.8		11.2		10.8		3.5	
9/28	22.8		10.6		11.4		3.5	
9/29	17.8		7.2		9.5		2.7	
9/30	21.4		7.2		10.4		4.4	
10/01	17.2		3.7		6.7		4.0	
10/02	18.6		2.8		7.0		3.6	
10/05	19.0		1.0		6.2		4.8	
10/06	-	+20	-		3.5	+20	3.4	
10/07	22.0		0.8	+20	24.1		6.2	
10/08	-		21.4		24.5		7.2	
10/09	20.2		21.4		21.4		6.4	
10/13	20.0		15.2		17.2		7.4	
10/14	22.5		-		19.6		9.5	
10/15	23.8		15.2		10.3		10.2	
10/16	26.4		15.5		20.0		10.0	
10/19	23.8		11.7		17.5		9.1	
10/20	30.2		12.8		22.0		13.4	

Analysis of the summer data in Table 7a demonstrates that the system was primarily photosynthetic and that the organic soil layer probably acted to buffer the system and provide nutrients. Some bacterial denitrification undoubtedly occurred.

The fall (low light conditions) test results in Table 7b indicate that bacterial denitrification had increased and that a substantial amount of nitrogen was being recycled into the system from the organic layer. It was concluded that this recycling may have occurred during the summer, but was not detected because of the high levels of nitrogen assimilated by the algae.

Effect of Biomass Regulation on Nitrogen Assimilation

The regulation of biomass during certain times of the year was found to be an important factor in Phase I. During Phase II, however, biomass regulation improved nitrogen assimilation only slightly, as indicated in Figure 45. This negligible effect of biomass regulation may have been due to the method of nutrient addition which caused the removal of more algae than inorganic sludge in the biomass regulation system. This assumption is borne out by the fact that when at the end of Phase II, iron was added by way of the biomass regulatory chamber instead of directly to the cultures, total nitrogen assimilation improved over the control unit.

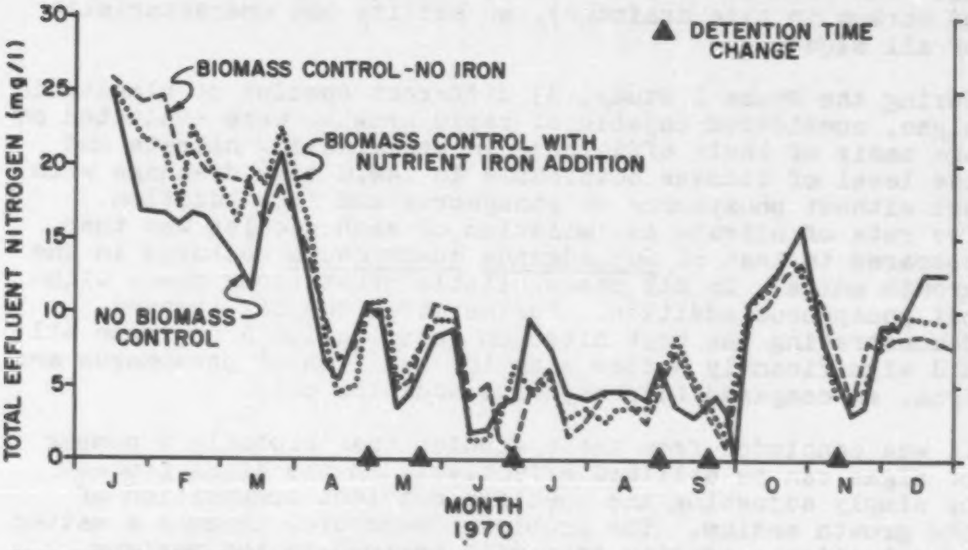

FIGURE 45-EFFECT OF BIOMASS CONTROL ON NITROGEN ASSIMILATION

Algal Species and Predation

The predominant algal species used in the Phase I investigation was the green alga, Scenedesmus quadricauda. Preliminary studies with this organism had indicated that it would grow in tile drainage, and in fact, it was often detected in the local water (though in small numbers). A ready supply of fairly large quantities of Scenedesmus was available from the University of California's Richmond Field Station. This particular genus also had other features that made it desirable as a test organism in tile drainage. It has been studied extensively and a large amount of information is available on its growth, nutrient requirements, separation properties, and potential use as a by-product. Specifically, it is one of the few algae whose nitrogen requirements have been studied. Scenedesmus quadricauda is known to use nitrate-nitrogen, the predominant form in tile drainage, as well as other nitrogen forms. In addition, Scenedesmus has a high specific growth rate and has been reported to use bicarbonate (the predominant form of carbon in tile drainage), an ability not characteristic of all algae.

During the Phase I study, 33 different species of planktonic algae, considered capable of rapid growth, were evaluated on the basis of their efficiency in assimilating nitrate and the level of biomass obtainable in IAWTC tile drainage with and without phosphorus or phosphorus and iron addition. The rate of nitrate assimilation of each species was then compared to that of Scenedesmus quadricauda cultured in the growth units. In all cases, little growth took place without phosphorus addition. Furthermore, the six species demonstrating the best nitrogen assimilation and growth all did significantly better with the addition of phosphorus and iron, as compared to phosphorus addition only.

It was concluded from these studies that probably a number of algae can be utilized effectively in the algal process by simply adjusting the specific nutrient composition of the growth medium. The problem, therefore, becomes a matter of selecting a species that will assimilate the maximum amount of nitrate in the shortest period of time and with a minimum of nutrient alteration. In this particular case, there was no apparent advantage in using a species other than Scenedesmus and this aspect of the investigation was not pursued any further.

During the first six months of 1970, Scenedesmus was the predominant species in the miniponds, as indicated in Table 8.

TABLE 8

PREDOMINANT ALGAL SPECIES DURING 1970 AT IAWTC

Minipond	Algal Species Present at Over Ten Percent of Total											
	January	February	March	April	May	June	July	August	September	October	November	December
1		S	S	S,D	S	S,D	S,D	S,D	S	S	S,D	S,D,U
2		S	S,D	S	S	S	S,D	S,D	S	S	S,U	S,U
3		S	S	S,D	S,D	S,D	D	D	S,D	D,L	D,U	U
4		S	S	S,D	S	S	S,H	S,H	S	S,U	S,U	S,D,U
5		S	S	S	S	S	D	D	D	S,D,U	S,D,U	S,D,U
6		S	S	S	S	S	S,D	S,D	S,D	S,D,U	D,U	D,U
7		S	S	S	S	S	S,D	S,O	S,O	S,O	S,D,U	S,D,U
8		S	S	S	S	S,O	S,D,O	S,O	U	U	S,D,U	S,U
9		S	S	S	S	S,O	S	S,H	S,U	S,O	S,D,U	S,D,U
10		S	S	S	S	S	S,O	S,H	S,D	S,D,O	S,D,U	D,U
11		S	S	S,H,L	O			U	S,U	S,H	S,H	S,D,U
12		C	C	H,L	H	S,E	S,D,U	S,D,H	S,D,U	S,D,U	H	H
13		S	S	S	S	S	S,D	S,D	S	S,D	S,D,U	S,D,U
14		S	S	S	S	S	S,D	S,D,U	S	S	S,D,U	S,D,U
15		S	S	S	S	S	S,D,O	S,H	S	S,D	S,D,U	S,D,U
16		S	S	S	S	S	S,D,O	S	S	S,H	S,U	S,D,U
17		S	S,D	S	S,C,D	S	S,D,H	S,D	S,D,U	D,U	S,U	S,D,H
18		S,D	S,D	S	S	S	S,D	D	S,D	S,D,H	S,D,U	S,D,U
19		S,C	S	S	S	S	S	S	S	S,D	S,D,U	S,D
20		S,C	S	S	S	S	S	S	S	S	S,U	S
21		P	P	P,H	P,H,N	H,U	H	H		P,H	H	H
22		H	H	H	H,U	H	H	H,U	H,U	U	H	H
¼-acre unit		S	S	S	S	S	S,D	S	S	S,D	S,U	S,D,H

S - *Scenedesmus*
C - *Carteria*
D - Diatom
P - *Phacus*
H - *Heteromastix*
L - *Lagerheimia*
O - *Oscillatoria*
N - *Nannochloris*
E - *Euglena*
U - Unidentified

On the other hand, during the same period, motile algae such as Carteria, Phacus, or Heteromastix were commonly encountered in the cultures not mixed. By mid-July and through the remainder of the year, a change was noted in algal species in the mixed units from predominantly Scenedesmus to a mixture of Scenedesmus, diatoms, Oscillatoria, and/or motile algae.

Since the species' changes in the mixed cultures were not observed until the units had been operated for some time, it was suspected that the sludge accumulation and probable decomposition had effected a change in general water quality and nitrogen forms.

As discussed in the Phase I report, usually some algal predators were present in most of the units, although usually in densities less than 500 per liter. It was generally observed over the three-year investigation that these numbers increased only after the unit had begun to fail for some reason. This suggests that the decomposition of algal material after pond failure releases cellular constituents which in turn stimulates predator reproduction.

Cellular Nitrogen

As nitrogen deficiency develops, the nitrogen content of Scenedesmus cells may decline from an original of 8 - 10 percent (dry weight) to as low as 2 percent (28). This decrease in nitrogen content is paralleled by a drop in chlorophyll (28). Syrett (35), working with nitrogen-starved cells of Chlorella vulgaris, found that (1) the addition of nitrogen resulted in marked changes in the respiration rate, (2) when both nitrate and ammonia-nitrogen were added to the medium, ammonia was assimilated four to five times faster than nitrate, and (3) assimilation of ammonia-nitrogen ceased when the available cellular carbohydrates were exhausted.

Richardson et al (70) found that with successive reductions in the influent nitrogen the percent cellular nitrogen dropped from 10 to 4 percent, while oxygen evolution, carbon dioxide uptake, chlorophyll content and tissue production were drastically reduced. They also found that the percent cellular nitrogen must drop to approximately 3 percent of the dry weight before an increase in lipid synthesis can occur. They concluded that all the nitrogen was bound in the essential cell constituents, and that the carbon fixed in photosynthesis was converted to lipids. Krauss (33) and others reported that when the ratio of carbon to nitrogen is low, nitrite or ammonia may be released into the medium. Conversely, at high nutrient carbon-to-nitrogen ratios, large amounts of intracellular carbon accumulate as carbohydrates or lipids (28, 29, 33).

The percent cellular nitrogen was found to be a good indicator of the nutrient balance in our system. The data plotted in Table 9 represent the average percent cellular nitrogen in algal cells grown under different operating conditions during 1970 at the IAWTC.

TABLE 9

AVERAGE PERCENT ALGAL CELLULAR NITROGEN FOUND UNDER DIFFERENT GROWTH CONDITIONS IN 1970

Unit	Percent Nitrogen	Unit	Percent Nitrogen
Phosphate added	7	No phosphate	8
Mixed	7	Not mixed	15
Soil	10	No soil	15
CO_2	8	No CO_2	10

These data indicate that the algae not receiving phosphate contained on the average of 1 percent more nitrogen than did the algae which did receive phosphate. The algae in cultures to which carbon was not added had 2 percent more cellular nitrogen than did comparable cultures that received carbon dioxide.

The data also show the difference between concentrations of cellular nitrogen of algae grown in units containing soil and those not containing soil, and between those in cultures which were mixed and those not mixed. Cultures grown in contact with soil consistently contained fairly low concentrations (50 to 100 mg/l) of motile green algae, such as Carteria, Heteromastix, Euglena, and Gonium. These algae had a higher percentage of nitrogen than did the algae in the control units. The algae were 75 percent protein, as opposed to a normal 50-percent level of protein. The presence of these particular types of algae, which are known to prefer reduced forms of nitrogen, may be considered to be a further indication that ammonia was being produced in the organic sludge layer. Moreover, it was also possible that some denitrification was taking place in the sludge-water interface, with the decomposing algal material providing the necessary carbon. However, the results of several analyses of the dissolved oxygen content of this zone showed that rather than being anaerobic, it contained dissolved oxygen at concentrations of 5 mg/l or more. A preliminary estimate of the cost of treatment in these units ("symbiotic process") showed that it could be as little as one-third the cost of the treatment processes described in Phase I. This low estimate prompted the proposal of a research investigation to define the mechanisms and costs involved.

Several studies were conducted which involved the use of the light box during Phases I and II to determine whether sustained low levels of nitrogen would be detrimental to the algae. An example of the typical algal response obtained in these studies is indicated by the series of curves in Figure 46. Normally, in these studies, the algae were cultured in tile drainage supplemented with phosphorus, carbon, and iron. The nitrate level in the growth medium was monitored and allowed to remain at zero concentration for varying lengths of time, after which the cultured medium was re-spiked with nitrate, phosphorus, and iron. As can be seen from the curves in Figure 46, the uptake of nitrogen by the algae was usually immediate even after five days, with 24-hour light, in a nitrogen-deficient medium. However, these tests do not necessarily represent what happened in the large test units, where, for example, many nutrients may be precipitated and settle out of the algal growth zone.

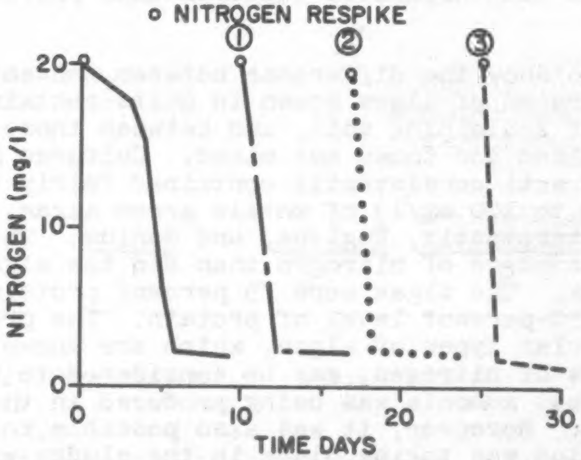

FIGURE 46-EFFECT OF A NITROGEN–DEFICIENT MEDIUM ON ALGAL NITRATE ASSIMILATION

Effluent Soluble Organic Nitrogen

The average differences in soluble effluent organic nitrogen during 1970 under various growth conditions are listed in Table 10.

TABLE 10

AVERAGE EFFLUENT CONCENTRATION OF ORGANIC NITROGEN IN VARIOUS TEST UNITS DURING 1970

Type of Unit	Organic Nitrogen (mg/1)
Mixed	
CO_2	1.0
No CO_2	0.7
Not Mixed	
CO_2	0.7
No CO_2	1.2
Detention Time	
Short	0.8
Intermediate	0.9
Long	1.2
Depth	
8 inches	0.8
12 inches	0.8
16 inches	0.7
Temperature	
Ambient	1.0
30°C	1.3

Inasmuch as the soluble organic nitrogen content of the effluent corresponds to changes in the influent nitrogen level (see Figure 3), most, if not all, of the soluble organic nitrogen appears to represent a fraction that is not readily biodegradable. Therefore, the small differences in average soluble nitrogen, as given in Table 10, may or may not have significance as applied to the overall system.

Operational Problems

During the month of May and again during July 1970 there was a period in which the nitrogen concentration of the effluent from the units increased as much as 10 mg/l in a few days. In July many of the cultures turned from a lush green to a dark brown color. The change in appearance was accompanied by a succession of algal species from Scenedesmus to diatoms and filamentous blue-green algae such as Oscillatoria. The increase in nitrogen content of the effluent in July occurred immediately after the ponds had been accidentally dosed with the herbicide propanil carried by wind drift from an adjacent field during a crop dusting operation. The herbicide was actually observed entering the test units. Not only was the herbicide actually seen, but also the weeds at the test site area exhibited the typical response to herbicides. However, a number of laboratory studies, as well as pesticide analyses conducted shortly after this occurrence, indicated that the pesticide was not responsible for the change in response, but that the occurrence was simply coincidental. Further tests indicated that neither light, temperature, change in detention time, water quality, nor the addition of carbon dioxide could have been responsible for the apparent failure.

In retrospect, spring characteristically was a period in which algal growth rates were at their maximum. The high algal growth rates led to high pH levels and dissolved oxygen concentrations which in turn were characterized by the accumulation of considerable amounts of organic and inorganic sludge in the test units. Furthermore, temperatures are at a maximum during the spring, with water temperatures often reaching 30°C. Since the rate of algal decomposition increases rapidly at high temperatures, the increase in algal nutrient regeneration could have accounted for the change in color as well as the increase in effluent nitrogen. To support these conjectures is the fact that the Scenedesmus cells were observed entering a zoospore stage, a stage normally associated with "shock" changes in the environment. In addition, the units left operating returned to their previous effluent nitrogen levels in from 2 to 15 days, also indicating an adjustment to a shock in the system. Most importantly, a comparison of the change in influent and effluent nitrogen in all the units during 1970 indicates that this increase in effluent nitrogen often coincided with an increase in the influent nitrogen or an increase in nitrogen loading as a result of detention time changes.

Pesticide Analysis

During 1970 when the storage pond was filled with tile drainage, samples were taken from the tile drainage sump, the storage pond and either the 1/4-acre demonstration pond or one of the miniponds and were forwarded to the Department of Water Resources water quality laboratory at Bryte, California, for pesticide analysis. The tile drainage was analyzed directly. Samples of algae from the test units were concentrated to an 85 - 90 percent cake and/or were oven dried before being analyzed for pesticide content. The results of these analyses are presented in Table 11.

TABLE 11

PESTICIDE ANALYSIS
1970

Date	Sample	Pesticide - parts per trillion (ppt)				
		DDT	Dieldrin	BHC	DCPA (Dacthal)	Parathion
3/70	Influent	*	10	*	*	*
	Test Unit	---	---	---	---	---
	Algae	4,000	*	*	*	*
5/70	Influent	10	*	12	155	44
	Test unit	27	*	*	55	*
	Algae	*	*	*	*	*
7/6	Influent	---	---	---	---	---
	Test unit	120	*	*	*	946
	Algae	340	*	*	*	*
7/15	Influent	3	14	*	*	76
	Test unit	---	---	---	---	---
	Algae	---	---	---	---	---
7/27	Influent	5	25	*	*	298
	Test unit	---	---	---	---	---
	Algae	9	*	*	*	*
8/24	Influent	170	15	*	*	*
	Test unit	240	*	*	*	*
	Algae	---	---	---	---	---
9/14	Influent	260	10	*	*	*
	Test unit	*	*	*	*	1200
	Algae	---	---	---	---	---
10/13	Influent	20	5	*	*	*
	Test unit	45	*	2	*	*
	Algae	31,000	*	*	*	*
11/20	Influent	50	3	*	*	*
	Test unit	125	*	*	*	*
	Algae	(570)1/	*	*	*	*

* - None reported.
--- - No sample.
1/ - Toxaphene.

Because the pesticide level tended to be low in the IAWTC tile drainage while that of the harvested algae at times was higher, the algae apparently did concentrate some of the pesticides, such as DDT. However, in several instances, the amount of pesticide in the algae concentrate was less than that in the influent water. It is possible that the high temperatures (20 to 30°C) and the high pH levels (8 to 11) in the mixed cultures may have been conducive to the degradation or volatilization of the pesticides. It is also possible that the efficiency of extracting pesticides from algal cells was low and that the analytical results do not reflect actual concentrations in the algae.

Evaporation During 1970

As previously described in the section on methods, a weather station was located at the IAWTC site to monitor climatic variations during 1970. Annual fluctuations in light and water characteristics were discussed previously. The net water loss by evaporation, which is evaporation minus precipitation, is shown for each month in Figure 47. As that figure indicates, the net loss of water by evaporation amounted to a significant total.

Data on net evaporation were not used in the interpretation, in the evaluation of nitrogen assimilation efficiencies, or in the Phase I estimates of the cost of treatment by the algal nitrogen removal process.

Diurnal Studies

Several diurnal studies were conducted during Phases I and II in which measurements were made of temperature, volatile solids, nitrate, pH, alkalinity, cell counts, dissolved oxygen (DO), and light penetration into the growth medium. As might be expected, some of these parameters varied as a function of the mixing regime. Variation in amounts of solids in suspension as measured by volatile solids, cell counts and packed cell volume, and in depth of light penetration varied with the mixing regimes of the different ponds. In those ponds with twice daily mixing, biomass in suspension peaked during the mixing period and decreased to a rather constant low level during the hours of darkness. Nonmixed ponds had little diurnal variation in effluent suspended solids. Cell counts at various depths in the same culture did indicate that some phototrophism occurred.

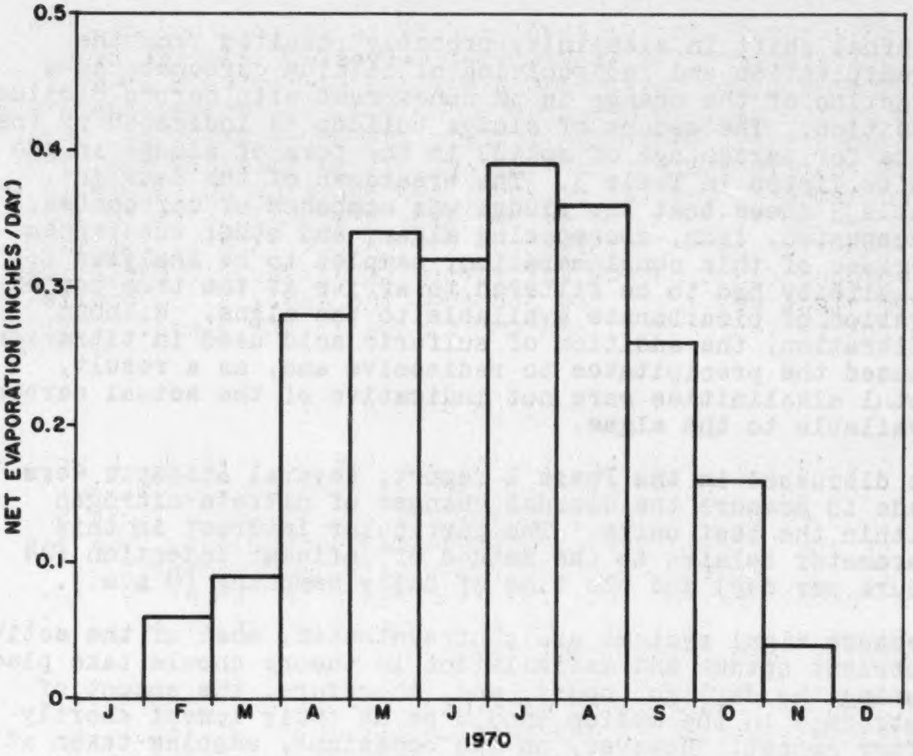

FIGURE 47-EVAPORATION MINUS PRECIPITATION-IAWTC,1970

Diurnal changes in carbon utilization (plotted as changes in alkalinity bicarbonate in Figure 27), in pH level, and in dissolved oxygen fluctuated with light intensity. Peak photosynthetic activity measured as high pH, high DO's, and low bicarbonate levels usually occurred from 2 to 4 p.m. The magnitude of these various parameters varied with the time of year and was a function of total light availability.

Determination of soluble orthophosphate during one diurnal study showed that as the pH increased, the concentration of orthophosphate decreased, probably because of precipitation. The relation between soluble phosphate and pH was plotted in Figure 32.

Another chemical constituent that was found to change over relatively short intervals of time was total alkalinity. The change should not have taken place when the influent concentration remained constant. Analysis of the total alkalinity in a completely mixed culture showed that the diurnal shift in alkalinity probably resulted from the precipitation and redissolving of calcium carbonate as a function of the change in pH concurrent with carbon dioxide addition. The amount of sludge buildup is indicated by the data for percentage of solids in the form of sludge in the units listed in Table 3. The breakdown of the data in Table 3 shows that the sludge was composed of carbonates, phosphates, iron, decomposing algae, and other substances. Because of this conglomeration, samples to be analyzed for alkalinity had to be filtered to arrive at the true concentration of bicarbonate available to the algae. Without filtration, the addition of sulfuric acid used in titration caused the precipitates to redissolve and, as a result, total alkalinities were not indicative of the actual carbon available to the algae.

As discussed in the Phase I report, several attempts were made to measure the diurnal changes of nitrate-nitrogen within the test units. The particular interest in this parameter relates to the method of influent injection (24 hours per day) and the time of daily sampling (8 a.m.).

Because algal systems are photosynthetic, most of the active nutrient uptake and assimilation in theory should take place during the daylight hours; and, therefore, the amount of nutrients in the medium should be at their lowest shortly after sunset. However, on two occasions, samples taken at various times through a 36-hour period did not show any measurable diurnal fluctuation in effluent nitrogen concentration. Perhaps the detention times being applied when these tests were made were sufficiently long to mask any fluctuations in nitrogen.

Operation Criteria -- 1970

Early in the Phase II studies, specific constant operational criteria were selected for the individual growth units (depth, nutrient addition, mixing, etc.). Only detention times were scheduled to be changed during the year. Only those factors shown to be important to maximum nitrogen assimilation in Phase I were included in the experiment design. The proposed operating schedule had to be interrupted in July 1970, when the algae in most of the outdoor units died. The miniponds with dead cultures were drained, cleaned, filled, and then inoculated with algae from those ponds still containing viable algal cells.

The effect of nutrient additions and mixing on maximum nitrogen removal is shown in Table 12. In general, the data show that additional phosphorus was required year-round, and that additional carbon was also required except during the months of July, August and September. The inorganic carbon requirement was apparently satisfied by either carbon dioxide or bicarbonate, although the use of bicarbonate caused a change in dominant algal species from those cultures receiving carbon dioxide. The effect of additional iron on maximum nitrogen removal was dependent on carbon addition. When carbon dioxide was added to the growth units, iron did not increase nitrogen removal, but was necessary when carbon was not added. Mixing (four hours per day, either daylight or night) was never necessary for maximum removal during the 1970 operational studies.

TABLE 12

EFFECT OF NUTRIENT ADDITION AND MIXING ON NITROGEN REMOVAL 1970 STUDIES

Month	Average Influent Nitrogen (mg/l)	Nutrient Addition				Mixing four hours per day
		PO_4-P	C[2]	Fe (without carbon)	Fe (with carbon)	
J[1]	29.6	+	+	+	-	-
F[1]	29.5	+	+	+	-	-
M[1]	29.5	+	+	+	-	-
A	26.9	+	+	+	-	-
M	24.7	+	+	+	-	-
J	16.5	+	+	+	-	-
J	13.0	+	-	+	-	-
A	13.1	+	-	+	-	-
S	14.4	+	-	+	-	-
O	19.5	+	+	+	-	-
N	23.8	+	+	+	-	-
D	20.6	+	+	+	-	-

[1] Units not under operational control.
[2] Carbon added for three hours per day.
+ Beneficial to maximum N removal.
- Not beneficial to maximum N removal.

The effect of depth and detention time on maximum nitrogen removal is illustrated in Figure 48. The influent nitrogen levels were adjusted to approximate the concentrations predicted for the San Luis Drain. The effluent values show the soluble nitrogen only, and are average values for the unit with maximum nitrogen assimilation during each period. The low percentages of nitrogen removal during the months of January, February and March were probably caused by a combination of factors including operational problems associated with a change of study personnel and low available light. Based on data from November and December, effluent nitrogen levels should have been on the order of 3 to 5 mg/l. Beginning in May, the effluent soluble nitrogen was consistently in the 2 to 4 mg/l range. In addition to the soluble portion, about 1 to 2 mg/l of particulate nitrogen (algal cells) would remain in the effluent from the harvesting processes.

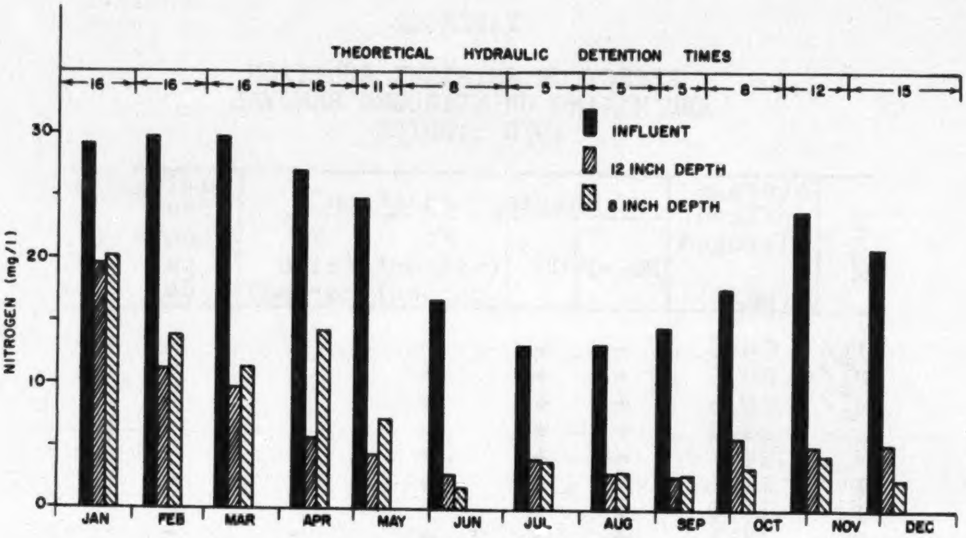

FIGURE 48 - EIGHT AND TWELVE INCH TEST UNITS WITH LOWEST EFFUENT NITROGEN CONCENTRATION DURING 1970

The detention times required for maximum nitrogen assimilation were on the same order as found in Phase I, that is, five days in the summer, and a maximum of about sixteen days during the winter months.

During most of the year, the 8- and 12-inch depth units produced comparable levels of effluent nitrogen, although from June through December, the effluent nitrogen concentrations from the 8-inch units were always equal to or slightly lower than those from the 12-inch units. From January through May, the deeper units consistently produced an effluent containing less nitrogen than the units operated at 8-inch culture depth. These results are apparently anomalous because any light inhibition of algae in the shallow units should have occurred during the period of maximum available light energy from June through July. The 1970 results differ from those of the 1969 studies when the shallow ponds had lower effluent nitrogen during the entire year, and especially during the winter months.

In addition to looking at effluent nitrogen levels, the factor determining the effectiveness of the removal systems studied at the IAWTC, the amount of nitrogen removed per unit of surface loading may be important in some applications of the algal process. The average number of grams of nitrogen removed per day per unit are tabulated in Table 13 (all units had equal surface areas). The data from this table have been plotted in Figure 49 and indicate that nitrogen removal per unit of surface area was greater at short detention times and the deeper culture depths.

TABLE 13

AVERAGE TOTAL SOLUBLE NITROGEN REMOVED
1970

Light (langleys/day)		100-400		400-600		600-800			600-400		400-100		
MONTH		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		Total Soluble N Removed (gm/day/unit) - Monthly Averages											
Depth	Detention Time												
8"	L[1]	1.3	2.5	2.8	2.0	3.8	4.5	4.5	5.0	6.7	4.9	4.1	3.1
	I[2]	1.6	2.4	3.8	3.5	5.9	7.5	8.3	7.4	6.4	6.2	5.6	3.9
	S[3]	2.5	2.5	3.6	3.2	7.0	10.6	18.7	12.0	6.9	6.4	5.5	3.9
	Average	1.8	2.5	3.4	2.9	5.6	7.5	10.5	8.1	6.7	5.8	5.1	3.6
12"	L	2.3	4.3	4.5	4.9	6.7	6.6	6.7	7.5	8.5	6.0	5.6	3.7
	I	2.8	5.3	5.6	6.5	8.3	9.2	9.1	9.6	8.3	8.0	5.5	4.8
	S	5.3	6.5	5.9	8.1	13.1	14.7	14.1	16.4	10.1	8.8	2.9	3.7
	Average	3.4	5.4	5.3	6.5	9.3	10.1	10.0	11.2	9.0	7.6	4.7	4.0
16"	I	3.4	3.4	4.3	7.1	11.2	11.2	12.7	12.5	10.5	9.1	5.5	5.3

[1] Long detention time.
[2] Intermediate detention time.
[3] Short detention time.

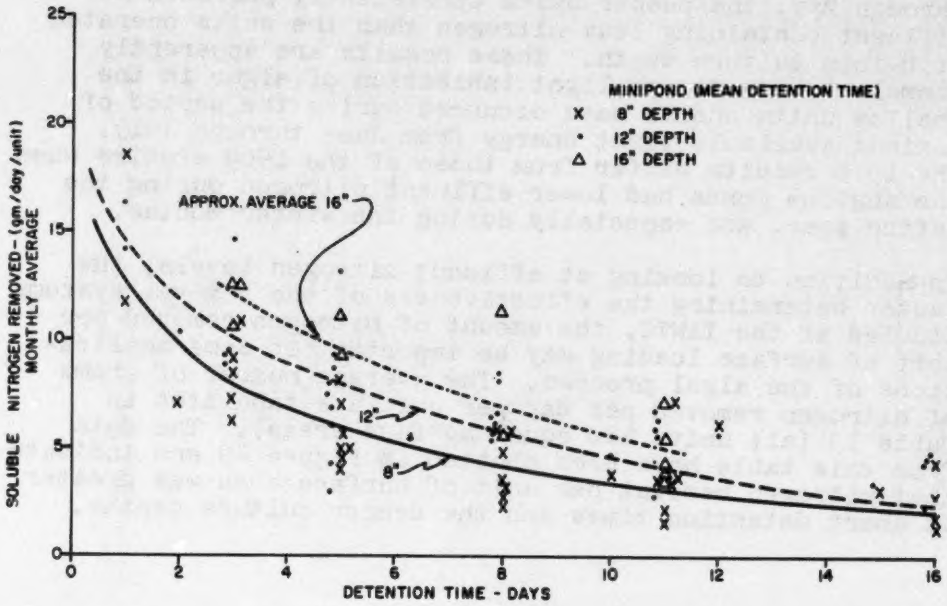

FIGURE 49-AVERAGE SOLUBLE NITROGEN REMOVED DURING 1970 AS RELATED TO DETENTION TIME AND DEPTH

The monthly changes in total nitrogen removal (gm/day/unit) at the three culture depths -- 8, 12 and 16 inches -- are plotted in Figure 50 and again illustrate that more nitrogen is removed per unit surface area in the deep ponds than in the shallow units, especially during the months with maximum available light energy. The practical implications of the greater removal efficiency (in terms of quantity removed per unit loading) in the deeper units can possibly be realized in a two-step nitrogen removal system. The first step involves the use of relatively deep (16 to 24 inches) ponds operated at detention times of 2 to 5 days. The effluent from these units could then be conveyed to shallower algal ponds for any necessary final polishing.

Using the data from Figure 50, it is possible to estimate the number of acres required to remove specified nitrate levels from agricultural tile drainage. Figure 51 shows such an estimate based on available light in the San Joaquin Valley, and a drainage flow of 300 cubic feet per second, the predicted peak flow for the San Luis Drain. For these

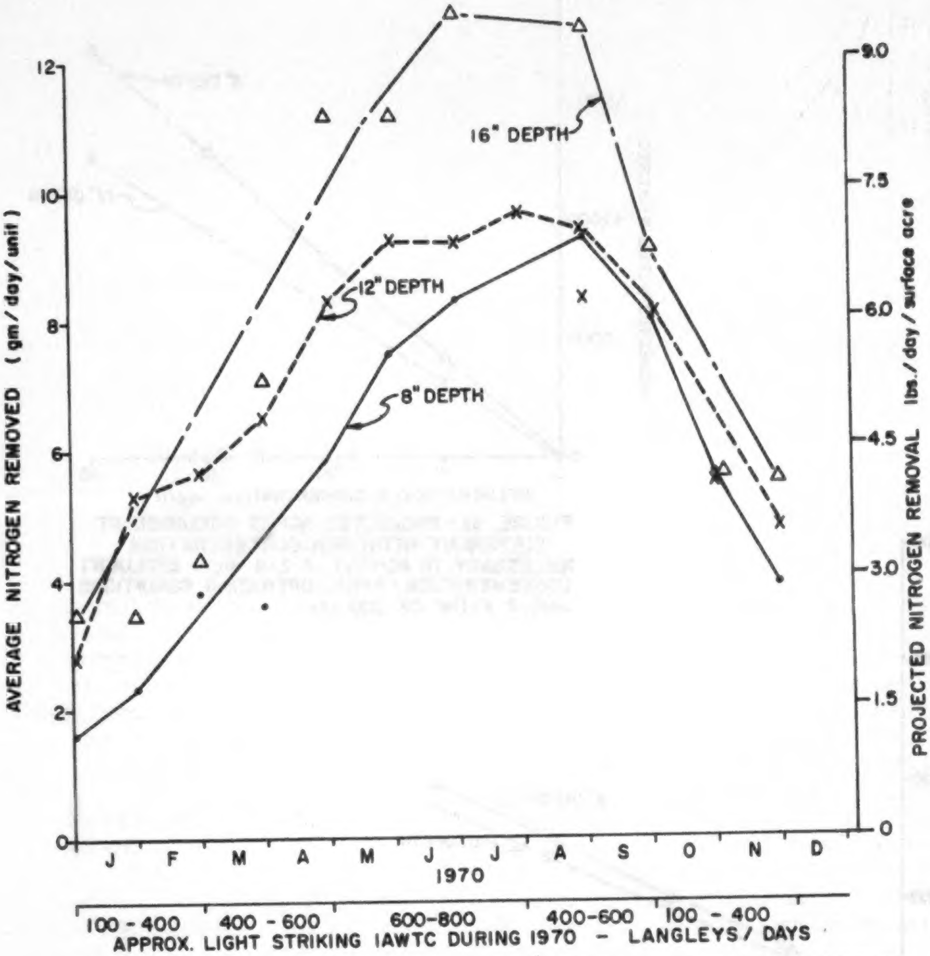

FIGURE 50-AVERAGE SOLUBLE NITROGEN REMOVAL AT VARIOUS DEPTHS IN INTERMEDIATE DETENTION TIME UNITS

conditions an estimated 8,000 acres of 12-inch deep ponds would be required to reduce 30 mg/l of nitrate-nitrogen to 2 to 4 mg/l soluble nitrogen. The same estimate made for August conditions, Figure 52, shows that only 6,500 acres of 12-inch deep ponds would be necessary to achieve the same level of nitrogen removal.

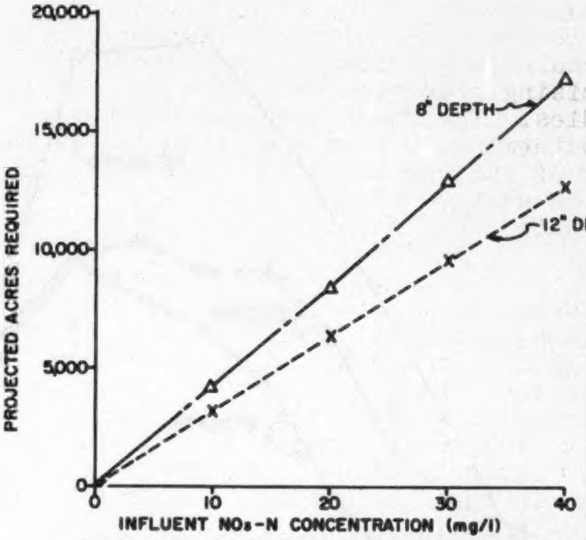

FIGURE 51 - PROJECTED ACRES REQUIRED AT DIFFERENT NITROGEN CONCENTRATION NECESSARY TO ACHIEVE A 2-4 mg/l EFFLUENT CONCENTRATION - APRIL OPERATING CONDITIONS AND A FLOW OF 300 cfs

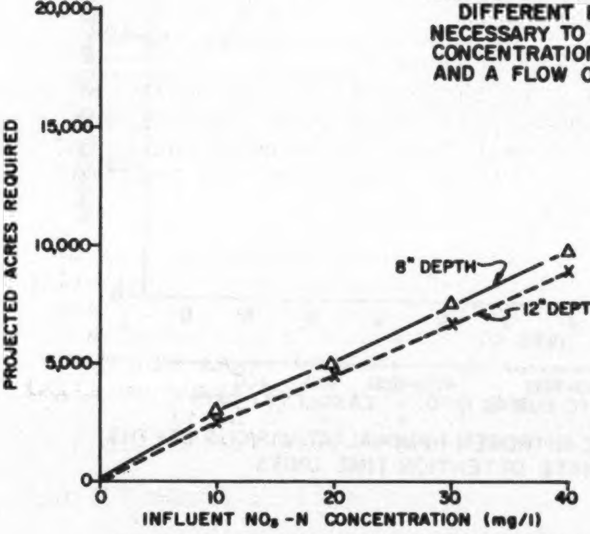

FIGURE 52 - PROJECTED ACRES REQUIRED AT DIFFERENT NITROGEN CONCENTRATION NECESSARY TO ACHIEVE A 2-4 mg/l EFFLUENT CONCENTRATION - AUGUST OPERATING CONDITIONS AND A FLOW OF 300 cfs

CHAPTER IV

ALGAL HARVESTING AND DISPOSAL

Algal Harvesting

During Phase I, many physical and chemical algae separation processes were screened and evaluated for their potential use in the algal nitrogen removal process. The chemical-flocculation-sedimentation method, along with several other promising processes, was selected for testing in the Phase II studies. The Phase II algae harvesting studies were designed to determine whether the operating requirements and efficiencies of the chemical-flocculation-sedimentation process varied seasonally during long-term operation and to further evaluate other selected separation processes.

As described in the Phase I report, algal harvesting was conducted in three stages based upon the percent algae (solids) obtained. The first stage, concentration, involved chemically or mechanically separating 90 percent of the algae from the growth medium and concentrating them to 1 to 4 percent solids. This was followed by a second stage, dewatering, which increased the slurry concentration to 8 to 20 percent solids. The dewatered algal mass was then dried in the final stage to 85 to 92 percent solids by weight. In this final stage, the dried algal product can be stored without decomposition (31).

As applied to the algal nitrogen removal process, the effluent from only the first stage of harvesting must be free of algae, because this is the effluent that will be discharged to the environment. If there were significant amounts of algae in the effluent from the second and third stages of harvesting, this effluent would then be recycled through the separation system.

During the Phase I studies, algal concentration was accomplished either by the coagulation-flocculation-sedimentation process or by rapid sand-filtration. The algal slurry was dewatered with the use either of a vacuum filter or of a self-cleaning centrifuge. The concentrated algal slurry was usually dried by air drying. (During the study an algal sample was successfully spray dried by the De Laval Separator Company at their spray-drying test facilities.)

Briefly, the processes (described in detail in Phase I) that proved to be effective were:

1. Shallow Depth Chemical-Flocculent Sedimentation (Concentration) - From 95 to 97 percent of the algae could be removed by this process at detention times ranging from 40 to 65 minutes. The resulting concentrate was slurry containing 1 to 2 percent algal solids. The amount of chemical-flocculent (ferric sulfate and/or Cat-Floc) required to bring about flocculation varied during Phase I. The variation in requirement was due to changes in algal growth as a result of operational or seasonal changes.

2. Rapid Sand Filter (Concentration) - This unit was operated on the basis of a design flow of 0.25 gpm per square foot to produce a 1 to 3 percent solid concentrate representing 95 percent or greater removal of algae.

3. Vacuum Filter (Dewatering) - Ninety percent of the algae from the effluent of the concentration process was removed by the vacuum filter. The resulting concentrate contained about 20 percent solids.

4. Self-cleaning Centrifuge (Dewatering) - The self-cleaning centrifuge dewatered the first stage concentrate to produce a paste having a solids content of about 10 to 12 percent (95 percent removal).

5. Algae Drying - Both air drying and spray drying proved to be effective methods of producing a product that could be stored without danger of deterioration.

The laboratory work, which was described in detail in the Phase I report, consisted of jar tests to determine the effectiveness of various mineral coagulants (lime, alum, and ferric sulfate) and many polyelectrolytes on algal separation. The theory behind coagulation, flocculation, and the use of polyelectrolytes was also covered in the report.

In Phase I, the average requirement for 90 percent algal removal, when ferric chloride had been added as a nutrient to a culture, was approximately 40 mg/l for lime, 20 mg/l for alum (aluminum sulfate), and 5 mg/l for ferric sulfate. In addition, approximately 60 polyelectrolytes were tested, singly and in conjunction with the three mineral coagulants. Seventeen of the polyelectrolytes were found to be comparable economically to the mineral coagulants. The cationic polyelectrolyte Calgon's "Cat-Floc" proved to be very effective. Almost complete removal was accomplished at less than 0.2 mg/l.

Because Phase II was primarily concerned with long-term operation, laboratory jar tests were routinely run to determine the seasonal variation of chemical requirements to obtain 90 percent removal of the algae. This included testing the mineral coagulants and the more promising polyelectrolytes to determine their economic potential under continuous operation. All the harvesting methods used in Phase II were described in detail in the Phase I report.

In addition to the laboratory evaluation of chemicals, the following separation studies were initiated:

1. Separation of algae by sedimentation in a shallow-depth sedimentation unit: the unit was operated continuously with effluent from the 1/4-acre unit. In this separation unit, chemical additions were applied as determined by the laboratory jar tests.

2. The effect of slurry depth on air drying: various bed materials were used in this study.

3. Flotation as a means of algal concentration.

Laboratory-Jar Tests

Polyelectrolytes. Listed in the Phase I report were 17 of the more promising of the polyelectrolytes tested, their effective range of concentration, and the mineral coagulants with which they were used. Because of the net negative charge on algal cells, only cationic polyelectrolytes proved to be effective flocculents. The polyelectrolytes were further evaluated in Phase II. In practice, because of the high costs of polyelectrolytes as compared to that of mineral coagulants, it would be more economical to simply increase the mineral coagulant concentration and omit the polyelectrolyte. The final evaluation of the polyelectrolyte-mineral-coagulant combinations showed their use would be more costly than with the mineral coagulants alone. However, it is recommended that future studies on chemical coagulation-flocculation and sedimentation should include an evaluation of cationic polyelectrolytes to determine whether the economics of their use would be competitive with the use of mineral flocculents under various growth conditions. Moreover, care should be taken as to the type of materials used as a coagulant with regard to the use the algae may be put. For example, Calgon's Cat-Floc is approved by the U. S. Public Health Service for potable water, but may not be approved if concentrated in food. The nitrogen contents of polyacrylamide and polyamide compounds were found not to be significant due to the low concentrations used. However, this could be an important factor in contributing nitrogen to the effluent if higher concentrations were required.

Mineral Coagulants. In 1970, routine jar tests were performed to determine the variations in optimal chemical requirements as a function of change in season. Figure 53 shows the average monthly cost (dollars per million gallons) for separation to 90 percent solids removal and down to 40 mg/l, as determined on the basis of results obtained in the tests. There were no determinations in July and no 40 mg/l studies in August. The values obtained for October are not representative because changes were made in the nutrient input to the culture. Table 14 presents the costs of 90 percent algal solids removal.

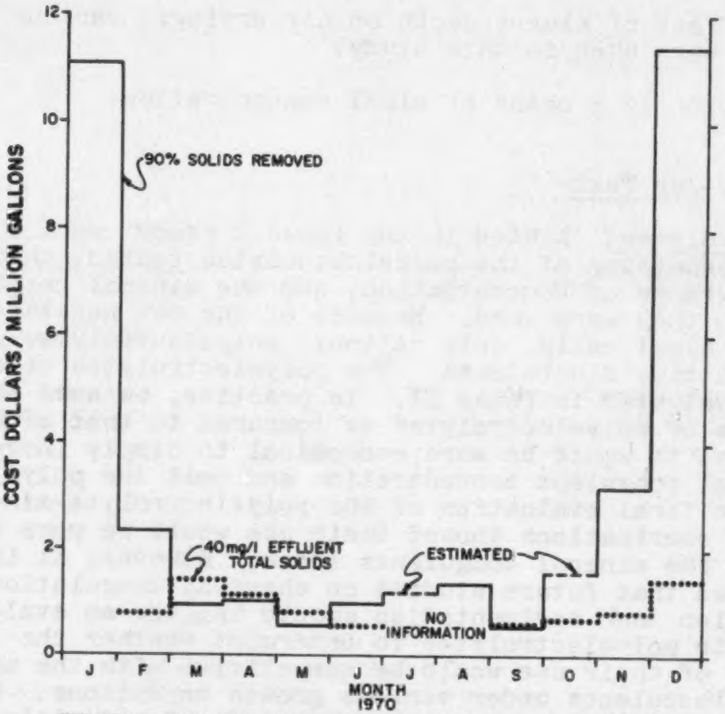

FIGURE 53-CHEMICAL COST REQUIRED FOR ALGAL CONCENTRATION

TABLE 14

ESTIMATED AVERAGE CHEMICAL COST PER MILLION GALLONS SEPARATED (1970) (JAR TEST DATA)

Year	Month	Dollars Per Million Gallons	Percent of Flow
1969	December	13.80	2.54
1970	January	11.10	2.54
	February	2.34	2.30
	March	0.94	8.46
	April	1.03	13.55
	May	0.56	14.00
	June	0.98	13.55
	July	1.17[1]	14.00
	August	1.37	14.00
	September	0.58	9.40
	October	1.60[1]	3.20
	November	3.16	2.46
	December	11.41	2.54

---

[1] Estimated.

According to the data plotted in Figure 53, the cost to remove solids to a residual of 40 mg/l is independent of original algal concentration. As discussed in the Phase I report, a concentration of 40 mg/l in the effluent was found to be about the maximum amount of allowable suspended solids that could be discharged to maintain the suggested maximum nitrogen content of 2 mg/l for discharge into the receiving waters.

Ferric sulfate was found to be the most effective coagulant tested in 1970. The only exception occurred when iron, chloride or sulfate was not added to the culture as a nutrient in December 1969, and in January and October of 1970. It was estimated from these data that the cost of chemical separation would probably be five to ten times greater when iron (1 to 3 mg/l) is not added to a culture as a growth nutrient.

Figure 54 shows estimates of costs for removing algae at optimum dosages of $Al_2(SO_4)_3$, of $Ca(OH)_2$, and of $Fe_2(SO_4)_3$ in terms of conditions prevailing during the 1970 runs.

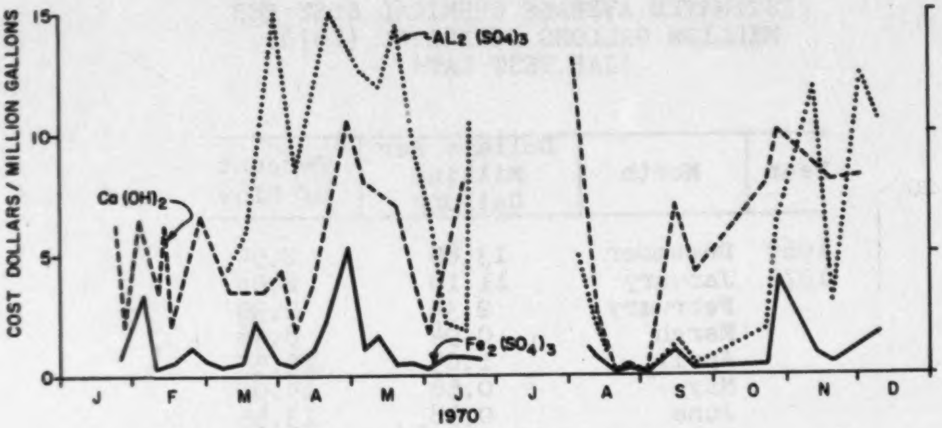

FIGURE 54 - CHEMICAL COST TO REMOVE TOTAL SUSPENDED SOLIDS TO 40mg/l DURING 1970

Although removal did not appear to be related to biomass concentration, some correlation was noted to degree of alkalinity. Ferric sulfate concentrations necessary for maximum solids removal were found to decrease with decreasing bicarbonate concentration, as is shown by the curve in Figure 55, in which required iron dosage is plotted as a function of alkalinity.

Operational Studies

Chemical-Flocculent-Sedimentation Unit. The shallow-depth sedimentation unit was continuously operated with the requirements for chemical flocculent additions generally determined by routine jar tests. The unit had a volume of about 130 gallons and was operated at theoretical detention times ranging from about one-half to one hour. In the jar test, one hour was the standard settling time. However, since in the sedimentation unit neither mixing velocity nor duration of mix could be varied, the actual efficiency of removal with chemical coagulation-flocculation and sedimentation achieved in the tests did not meet expectations. The costs in terms of dollars per million gallons of culture processed as based on performance of the sedimentation unit are plotted in Figure 56. The chemical costs alone usually ranged from one to four dollars per million gallons treated.

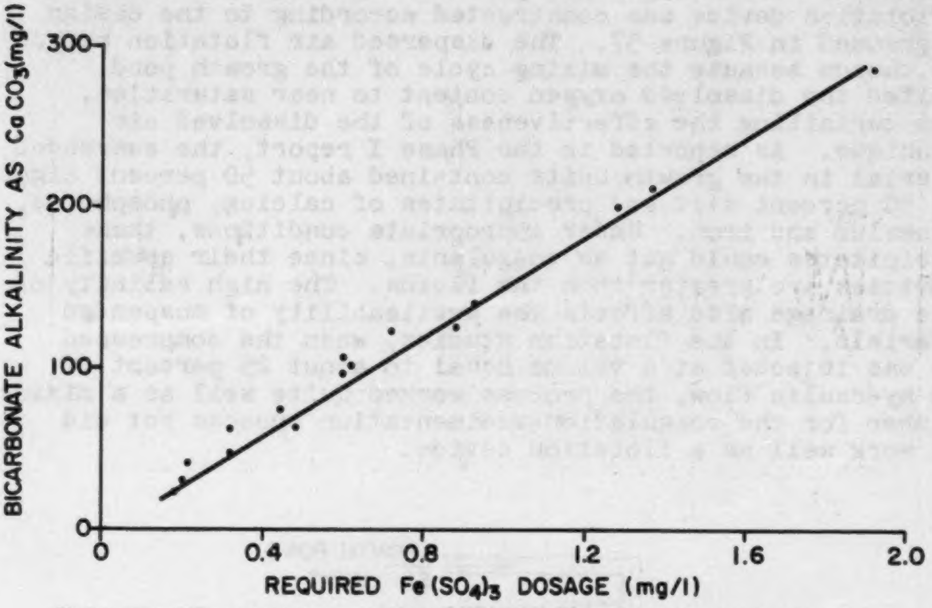

FIGURE 55 - EFFECT OF BICARBONATE ALKALINITY ON IRON SULFATE REQUIRED TO REDUCE SUSPENDED SOLIDS TO 40 mg/l.

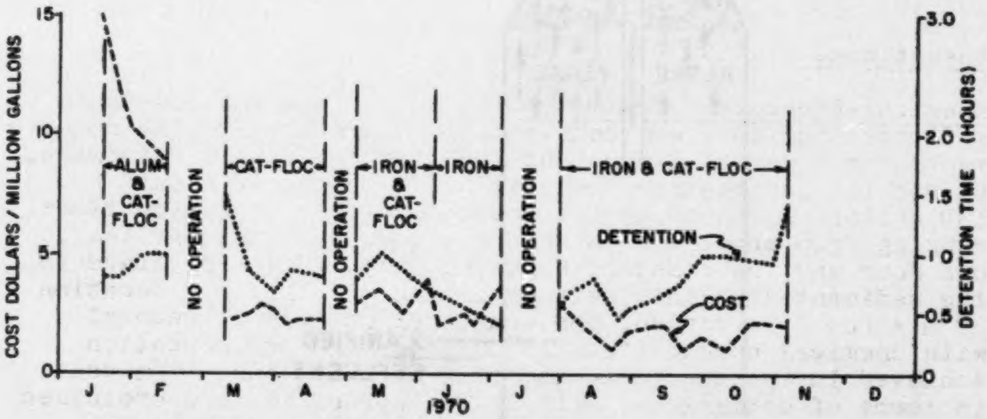

FIGURE 56 - COST OF SEPARATION IN SHALLOW DEPTH SEDIMENTATION UNIT DURING 1970

Flotation. To determine the practicability of flotation as a means of harvesting algae grown in agricultural wastewater, a flotation device was constructed according to the design diagrammed in Figure 57. The dispersed air flotation method was chosen because the mixing cycle of the growth pond limited the dissolved oxygen content to near saturation, thus curtailing the effectiveness of the dissolved air technique. As reported in the Phase I report, the suspended material in the growth units contained about 50 percent algae and 50 percent silt and precipitates of calcium, phosphorus, magnesium and iron. Under appropriate conditions, these precipitates could act as coagulants, since their specific gravities are greater than the fluids. The high salinity of tile drainage also affects the settleability of suspended materials. In the flotation studies, when the compressed air was injected at a volume equal to about 25 percent of the hydraulic flow, the process worked quite well as a mixing chamber for the coagulation-sedimentation process but did not work well as a flotation device.

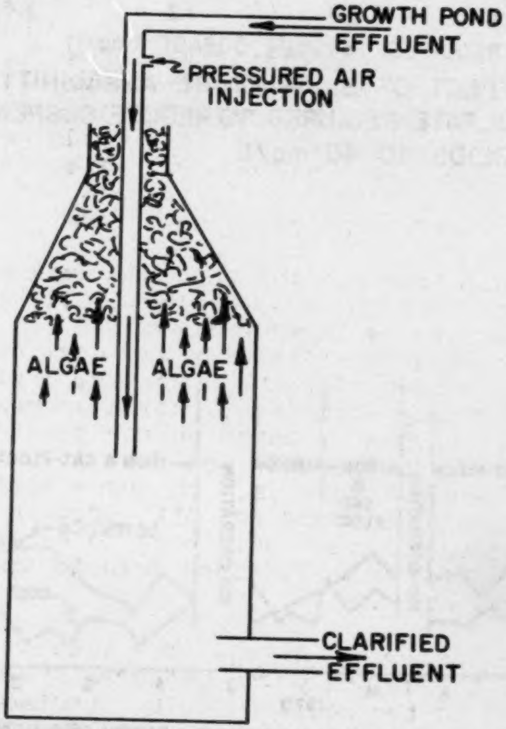

FIGURE 57 - SCHEMATIC OF FLOTATION CHAMBER TESTED AT IAWTC

From observations made in the study, the flotation-separation process probably would not be practical unless the percent of volatile solids could be increased in the growth phase of the process.

Air Drying. Costs of drying bed construction, methods of collecting the dried algae, and the use of the product determine the type of material to be used in building a drying bed. One of the procedures tested for processing algal paste after its concentration and dewatering was air drying. During Phase II, several studies were conducted to determine whether sun drying would be economically comparable to other methods -- as, for example, spray drying. Four types of surfaces -- sand (wet and dry), cloth, black plastic, and asphalt -- were tested as a substrate on which to spread the algal paste. The main criterion was the permissible loading rate of algal slurry per unit surface area. As is to be expected, increased radiation permitted greater loadings than did increased initial solids concentration; that is, more energy input on the one hand, and on the other, less water to evaporate. A dry sand bed and shallow slurry depth proved to be a most effective method of drying, but it resulted in a poorer grade product. Cloth as a drying surface was the second in terms of efficiency. A disadvantage in the use of cloth is handling it in a large-scale operation. Observations made in the tests gave reason for concluding that an asphalt surface would be the most practical, since the algal paste could be applied easily and the dried product could be readily scraped off. Moreover, the product would be a high grade one.

In terms of permissible loading rate per unit of surface area (except for the sand bed), maximum efficiencies were attained when the sludge layer was applied 1.5 inches deep. However, the increase in efficiency was negated somewhat by the longer time required for the necessary drying. At the time of maximum drying efficiency, which corresponds to the time of heaviest loading as based on the predicted drain flow, the required slurry depth would be 1.25 inches, and the drying time would be 3 days. In those operations, in which preservation of vitamin and high protein quality is essential, the deeper layer would have to be abandoned in favor of a layer less than 0.5 inch deep to complete drying in one day or less.

Differential Separation of Algae. The data on chemical costs discussed in the preceding paragraphs were based on results obtained in the shallow-depth, chemical-flocculent-sedimentation unit normally operated on-line 24 hours per day and from processing algae from the 1/4-acre demonstration unit. The influent for the process was pumped from the mixing pump sump (Figure 6), an area where large quantities of inorganic and organic sludge tended to accumulate. Analyses of the effluent solids from the separation unit showed that they usually were only about 50-percent volatile. The nonvolatile portion mainly comprised precipitates of carbonate, phosphate, and iron.

A 3,000-gallon capacity, incidental sedimentation unit was operated. It was not preceded by a chemical flocculent. It also was used with the 1/4-acre demonstration unit and was used primarily as a biomass regulation tank. The influent to the tank also came from the sump for the mixing pumps. It was passed through the tank at a rate which resulted in theoretical detention times of from 2 to 3 hours. The effluent line on this unit was modified with a U-tube device. Effluent could be taken from the device at various depths. The effluent device was installed after the algal portion of the suspended solids was observed to settle differentially according to percent volatile solids, presumably because cell density increased with age. Depending on the depth from which the effluent was drawn, up to 90 percent volatile solids could be returned to the 1/4-acre demonstration unit. The high volatile solids portion was thought to contain the younger, actively assimilating cells, which are desirable for algal high-rate growth systems. Several times each week the flow to the biomass regulatory tank was stopped, the supernatant was fed by gravity back to the 1/4-acre unit, and the settled sludge was removed. Analysis of the sludge material showed that it was about 50 to 60 percent mineral and clay material and 40 to 50 percent algae.

Sludge Accumulation in the 1/4-acre Pond. Although the biomass regulation tank helped to reduce the total solids in the 1/4-acre pond, much of the settleable material settled within the pond and never reached the sedimentation tank. It soon became apparent that sludge was rapidly accumulating in the 1/4-acre demonstration unit. The accumulation led to a reduction in the amount of light reaching the algal cells, and hence to a deterioration in the performance of the pond. An attempt was made to reduce the total nonassimilating solids in the test unit by pumping out sludge from the areas of greatest sludge accumulation. This proved to be only a temporary solution. Because of the design of the pond system, the problem of unwanted sludge accumulation proved to be a difficult one to solve.

As mentioned earlier, iron normally was added as batch doses to the individual test units. Because iron is an excellent algal flocculent (and was probably responsible for much of the settling in the test units), its addition to the 1/4-acre unit was stopped. Within several days the color of the pond culture turned from a lush green to a very peaked "washed-out" color, the amount of algae in suspension decreased, and the amount of chemical required for separation increased. These reactions were not expected, inasmuch as sludge in the pond contained large amounts of insoluble iron. Apparently, the residual iron in the pond was in a form unusable by the algae. This must have been the case because, when iron addition was resumed, there was an immediate beneficial response in terms of: (1) increased numbers of algae in suspension; (2) a decrease in required chemical dosages for separation and, consequently, a decrease in cost of separation; (3) a change in color back to lush green; (4) an increase from 40 - 50 percent to 60 - 70 percent in the volatile solids content of the pond effluent; and, most importantly (5) more nitrogen assimilation.

When iron addition was resumed, the iron was added to the biomass regulatory tank rather than directly to the growth unit. Monitoring of the influent to and the effluent from the biomass regulatory tank showed that although most of the iron added to the tank remained in the tank and helped to settle nonorganic solids, the amount, although small, of soluble iron reaching the pond was sufficient to meet the needs of the algae. The dissolved iron concentration in and out of the biomass regulation unit leveled off at about 0.5 mg/l.

A similar addition of iron to the biomass regulatory tank of one of the 1,000-gallon test units showed similar results, particularly in terms of increased algal suspension.

Algal Disposal

No experimental work was done in either Phase I or II on the disposal or potential use of algae as a by-product of the removal of nitrogen from tile drainage through the culture of algae. However, a discussion of these aspects is contained in the Phase I report. The discussion was based on information gained from a search of the literature. Briefly, because of their high protein content, algae appear to have a potential use in this country as (1) an animal food supplement, (2) a soil conditioner, (3) a food for the rearing of organisms in commercial aquaculture, (4) a raw material for certain drugs, (5) a source of inorganic and organic chemicals, (6) a raw material for adhesives, and (7) a food for direct human consumption.

Work presently being carried out in underdeveloped countries (71, 72, 73) indicates that there is a potential for the immediate use of algae as a by-product of wastewater treatment. In fact, in certain portions of Southeast Asia, both fresh water and marine algae are presently being grown commercially with artificial growth media for direct human consumption.

Projections of algal production for the proposed algal-nitrogen removal plant are shown in Table 15. As indicated, up to 83,000 tons of dried algae would be produced annually. If sold as a protein source, the monetary returns could amount to eight million dollars per year, an amount which could be credited to the cost of treatment.

TABLE 15

ESTIMATED ALGAL PRODUCTION BY
AN ALGAL STRIPPING PLANT, 1975-2000

Year	Tons of Algae per Year		Approximate Value as Substitute for Soybean Meal	
	Maximum	Minimum	Maximum	Minimum
1975	13,300	8,410	$1,330,000	$ 845,000
1980	27,200	18,000	2,720,000	1,795,000
1985	44,300	29,610	4,430,000	2,965,000
1990	62,000	42,400	6,200,000	4,240,000
1995	75,300	54,100	7,530,000	5,410,000
2000	82,610	65,510	8,265,000	6,555,000

During Phase II, several samples of algae, soybean meal, and cottonseed meal were analyzed for their amino acid content. The results of the analyses are shown in Table 16. The algae samples had a lower amino acid content than did either the soybean or the cottonseed meal. However, the algae samples contained only about 50 percent volatile material. A large part of the algae samples was nonorganic. As stated earlier, the percent volatile solids, as well as that of the cellular nitrogen, can be varied by applying suitable operational procedures.

TABLE 16

AMINO ACID ANALYSIS
(micromoles/mg sample)

Amino Acid	Soybean Meal	Cottonseed Meal	Algae 1*	Algae 2*	Algae 3*	Algae 4**
Lysine	.231	.131	.052	.107	.085	.033
Histidine	.097	.085	.017	.027	.030	.011
Arginine	.205	.281	.042	.110	.093	.048
Aspartic Acid	.465	.289	.099	.183	.177	.073
Threonine	.17	.117	.052	.086	.082	.041
Serine	.249	.173	.055	.097	.092	.044
Glutamic Acid	.668	.561	.100	.202	.161	.077
Proline	.237	.142	.055	.083	.082	.039
Glycine	.295	.230	.108	.209	.189	.076
Alanine	.253	.177	.125	.183	.164	.071
Half Cystine	.032	.032	.005	.009	.0056	
Valine	.194	.142	.056	.104	.091	
Methionine	.038	.033	.015	.027	.026	.013
Isoleucine	.165	.094	.034	.064	.058	.030
Leucine	.301	.186	.077	.131	.125	.052
Tyrosine	.072	.053	.017	.045	.045	.015
Phenylalanine	.149	.129	.032	.060	.059	.032

* - Algae from mixed unit.
** - Algae from nonmixed unit.

CHAPTER V

PROCESS EVALUATION

Summary of the Phase II Investigation

The Phase II operational studies confirmed the year-round technical feasibility of stripping nitrogen from tile drainage using algae. Several major changes in operating conditions were observed during prolonged unit operation. These changes were significant enough to warrant a reevaluation of the process in terms of design and cost estimates.

Probably one of the most important changes from Phase I in basic operating criteria was the discovery that mixing was not required for maximum nitrogen removal. An analysis of mixed and nonmixed cultures, both with and without carbon supplementation, showed that the only difference in total nitrogen assimilation between cultures was that which was due to the adding of carbon dioxide by way of the mixing pumps. Results of a special study involving a culture that was not mixed but which received supplemental carbon dioxide via a gas diffuser confirmed that mixing was not required. Furthermore, at the end of May 1970, when the influent nitrogen concentration decreased to a level at which carbon supplementation was not required, all the comparable mixed and unmixed cultures assimilated approximately equal amounts of nitrogen.

A further comparison of mixed cultures with cultures not mixed indicated that mixing could have been detrimental to the system in that it: (1) reduced the amount of light available to the algae because of the turbidity imparted by the re-suspension of nonphotosynthesizing material, (2) decreased nutrient solubility through reactions accompanying aeration, and (3) probably increased the recycling of nitrogen into the system from the sludge. However, because of the design of the growth units used in this study, mixing was the only method of adequately removing solids from the units.

The accumulation of large amounts of sludge under prolonged unit operation was another major factor that was not evident in the four- to six-week studies conducted during Phase I. The units in Phase II were originally scheduled to operate for one year but because of operational problems in July 1970, most of the units were drained, cleaned, and restarted; thus there were two distinct runs, one of about six months and one of about five months. Measurement of the sludge accumulation in Phase II indicated that a substantial portion

Preceding page blank

of the phosphorus, carbon, and iron became tied up in an unassimilable form in the test units within a matter of several months. This sludge buildup was thought to have detrimental effects other than those previously mentioned. As temperatures increased during the summer, algal decomposition was accelerated and there was a resulting increase in release of ammonia from the sludge. Results obtained from adding ammonia, the major nitrogen form released during algal decomposition, to one culture, indicated that it was preferentially assimilated with respect to nitrate-nitrogen. It therefore seems likely that the algae could utilize ammonia released via nitrogen regeneration in the test units under periods of high temperatures.

During Phase II, the availability of light to the algae and nitrogen loading was found to be the most important factor affecting nitrogen assimilation. A comparison between the effluent nitrogen concentrations from test units in which the cultures had equal surface area but were maintained at different depths showed that, although the effluent from cultures operated at the 8-inch depth usually had a lower nitrogen content than that from comparable ones held at a 16-inch depth, when compared in terms of total nitrogen assimilated, the deeper unit was often much more efficient.

Relating total nitrogen removal to nitrogen loading and to operating depths at different light energy levels indicated that these two factors combined to determine the performance of a culture. Extrapolation of all the 1970 operating data for unit depth indicates that, when total light per day is at 600 langleys, the minimum pond depth should be 24 inches; at 300 langleys, it should be 16 inches; at light energy levels less than 300 langleys, the recommended depth is 12 inches. In addition to looking at unit efficiency in terms of grams nitrogen removed per unit surface, some consideration must be given to effluent concentration since waste discharge requirements often specify allowable limits of individual components. Using effluent nitrogen concentration as a criterion, the 8-inch ponds provided an effluent which was most consistently close to the 2-mg/l limit suggested in a 1967 Federal Water Pollution Control Administration report (9).

The total nitrogen that could be removed by the algal system per unit of time was found to be directly proportional to the influent nitrogen loading and total light available to the algae. The amount of light available to photosynthesizing algae was found to be reduced when the accumulation of sludge reached a point at which its re-suspension imparted an excessive turbidity to the culture medium.

The shading of the cultures by pond walls and the resulting light available to the algal cultures were not considered in the Phase I studies because all but two of the units were operated at the same depth. The difference in available light between ponds with 18-inch walls as compared to those with 10-inch walls was considered significant. Some theoretical wall shading estimates based on sun angle were made in Phase II. These estimates showed that up to 30 percent of the available direct sunlight may have been blocked from the units during the winter months. No attempt was made to estimate the effect of walls on diffused (scattered) light, but the figures do indicate that the small-unit data may be conservative estimates of nitrogen removal rates in large ponds where the effect of shading would be minimal.

Another important aspect of algal nitrogen assimilation noted in the Phase II studies was that low operating temperatures (10 to 15°C) were not necessarily growth limiting. A comparison of the performance of a culture functioning at summer temperatures (25 to 30°C) with that of a control culture growing at the ambient temperatures prevailing during the fall of 1970 showed that there was no difference in nitrogen assimilation within the temperature range covered in the comparison (15 to 30°C). The temperature effect noted in the Phase I studies is now thought to be the result of changes in total light energy reaching the cultures, rather than of changes in temperature. Probably, temperatures above 25°C were somewhat detrimental to the net nitrogen removal efficiency of the system because of their accelerating effect on the decomposition of bottom sludges and subsequent release of algae nutrients tied up in the sludge.

The changes in detention times made in the Phase II studies were based on Phase I data. After prolonged operation, they were found not to bracket the optimal detention times necessary for maximum nitrogen removal during most of the year. In the Phase II studies, cultures operated for a period of two weeks at detention times of two days during mid-summer assimilated 90 percent of the influent nitrogen, whereas in the Phase I studies a minimum of five days was indicated. During the winter period in Phase II, when light was minimal, a 15-day detention time was found to be adequate for the production of an effluent having a soluble nitrogen content of 4 to 5 mg/l.

The nutrient studies during Phase II confirmed that some supplemental carbon, phosphorus, and iron would be required throughout the year. In fact, all the studies indicated that maintenance of maximum nitrogen assimilation depended on a completely balanced nutrient system.

Early in the spring, when carbon was the limiting nutrient in the cultures, as much as 10 mg/l nitrite-nitrogen was found in the pond effluent. When a sufficiently large supplement of carbon was introduced into the cultures, an immediate decrease in nitrite-nitrogen took place. The decrease implies that although nitrate uptake or cellular reduction to nitrite may not be affected by carbon deficiency, the further reduction of nitrite to ammonia is strongly dependent on an adequate supply of carbon.

The carbon-to-nitrogen ratio required in the growth medium proved to be 5 to 1, a ratio which is in agreement with the ratio of the two major nutrients in the algal cells. It was found that when the ratio of carbon to nitrogen was lower than 5 to 1, the addition of either carbon dioxide or bicarbonate was a satisfactory method of restoring the correct rates and achieving the required nitrogen removal efficiencies. The addition of bicarbonate did appear to cause the algal species' composition to change, but the change did not appear to adversely affect nitrogen removal. The total alkalinity of the influent was found to be a good indicator of carbon availability, although neither air-water exchange nor insoluble carbonate resolution was considered. In a practical operation, at times of high nitrogen concentration when supplemental carbon is required, carbon dioxide could be injected into the unit for two to three hours per day or possibly bicarbonate could be mixed into the influent.

Both phosphorus and iron were found to be required at the IAWTC throughout the year, although the exact amount of the two elements available to the algae was not determined. The phosphorus dosage to the cultures was based on a phosphorus-nitrogen ratio of 1 to 10, which is about that of the algal cell. Usually a small amount of phosphorus was present in the effluent. About 90 percent of the incoming phosphate was removed by passage through the cultures, by way of algal assimilation and/or precipitation.

The addition of iron to cultures receiving carbon dioxide had no significant effect on nitrogen; however, the addition of the same amount of iron to cultures not enriched with CO_2 caused a significant increase in nitrogen removal. The CO_2 evidently enhanced the availability of iron to the algal cultures. The method of adding iron (as $FeCl_3$) left much to be desired and needs more study.

A comparison of the cultures grown in contact with soil with one grown in the absence of soil under comparable conditions (that is, not mixed) indicated that the mechanism of nitrogen removal was probably the same in both cultures. The results obtained in several in situ studies with cylinders from which light was excluded from some and admitted to others indicated that an algal-bacterial symbiotic system probably was involved in nitrogen removal. The extent to which each of these groups of organisms contributed to the overall system was found to vary with light availability. Tests conducted in the spring and summer indicated that the system was probably 90-percent photosynthetic, although the bacterial portion probably was contributing to the overall process. In the fall, nitrogen removal was mainly bacterial, with the bacteria probably using the degradable carbon released from the decomposing algae that had accumulated during the previous portion of the year. Another point of interest noted in the symbiotic studies was the recycling of nitrogen from the sludge-organic layers back into solution. This nitrogen recycling was thought to occur in all the algal and symbiotic test units in varying degrees, and probably had a significant effect on overall efficiency of operation during 1970.

The 1970 studies on the harvesting of algae demonstrated that 90 percent or more of the algae in suspension could be removed throughout the entire year in a continuous operation by the chemical-flocculent-sedimentation process. However, the level of chemical addition required to accomplish this removal was found to depend on a number of algal growth factors. In all cases, the cost of the chemicals would be negligible in relation to overall treatment costs.

During the 1970 studies, algae assimilated enough nitrogen to maintain a year-round effluent nitrogen concentration of about 2 to 5 mg/l. This does not include the nitrogen content of the algae not removed in the separation process (about 5 percent), which would probably add 1 to 2 mg/l nitrogen, although it does include the nondegradable dissolved organic portion. Projections using actual nitrogen removal rates during Phase II indicate that it should be possible to remove virtually 100 percent of the influent-soluble nitrogen when some of the operational problems are resolved.

Cost of Treatment

As stated in the Phase I report, this was a preliminary investigation and was not designed to provide definitive costs data. However, analysis of the data collected during the Phase II operational investigation indicated that the two primary factors affecting the efficiency of the algal system are: (1) the light available to the algae, and (2) the influent nitrogen loading. If these two factors are known, the area required for treatment can be derived, thus providing a basis for calculation of treatment costs.

CHAPTER VI

PROPOSED ALTERNATIVE TO THE MIXED-REACTOR SYSTEM STUDIED IN PHASES I AND II

Logically, the next step in the nitrogen removal studies will be to construct a pilot plant operating on the combined flow of several tile drainage systems. The selection of the specific processes to be studied, either algal or bacterial, will depend on several factors including available time and money. The purpose of this section is to provide some details on an alternative algal system to that proposed in the Phase I report. Although there are no experimental data to verify the reliability of the proposed "slug-flow" system, some theoretical considerations indicate that preliminary testing would be beneficial.

The Phase I design proposed to meet the operating criteria determined in the early stages of the investigation basically considered of a number of high-rate growth units clustered around a central pumping plant. Four hours of mixing per day in each unit could be accomplished by directing the unit's flow to the central pumping plant via an elaborate network of concrete pipes and automatic valves. In addition to the pond structure, each unit was to have a 200 x 130 foot sedimentation basin lined with concrete. The basin was to be 10 feet deep at the influent end and 15 feet deep at the effluent end, and to be supplied with chain-driven scrapers to remove the sludge. Basically, the proposed system was of the same configuration as that of the test units investigated at the IAWTC.

The Phase II investigation indicated that algae cultured in a "stirred reactor" for any length of time probably would be under unfavorable growth conditions, which could limit the ultimate nitrogen removal potential of the process. Probably the greatest hindrance to the nitrogen removal activity of the algae would be the curtailment of light penetration by the accumulation of a nonphotosynthetic sludge on the bottom of the pond. As shown in Figure 58, it is predicted that light and nutrients would remain above limiting conditions until near the effluent end of a "slug-flow" system. Light and nutrient limiting conditions to the algal culture were the case in the stirred reactor used in the IAWTC studies. As McCarty et al (70) have observed, algae grown in the nutrient-deficient conditions release organic material into the medium. This material can affect algal growth and aging. Conversely, the basic premise of a slug-flow system is that all variables will be in excess until near the effluent end of the unit, at which time one will become limiting -- nitrogen, for example.

Preceding page blank

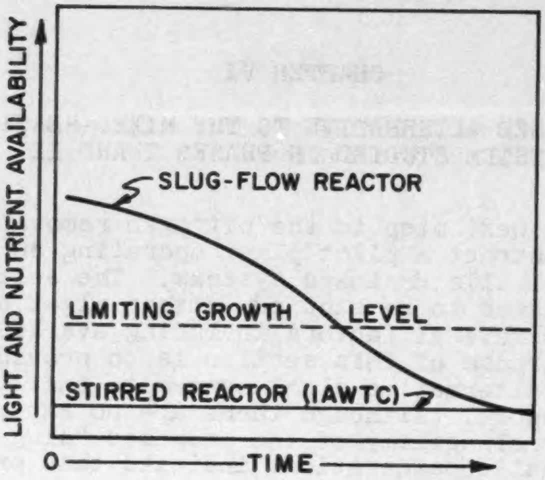

FIGURE 58 - THEORETICAL NUTRIENT AND LIGHT AVAILABILITY TO ALGAE IN TWO TYPES OF ALGAL REACTORS

Another limitation of the stirred reactor system used at the IAWTC is that the algal population is in all stages of growth rather than entirely in exponential growth (Figure 59). Because auto-flocculation as well as inorganic precipitate buildup is primarily a function of culture age, high pH values, and nutrient deficiency (and these conditions were all encountered), a considerable buildup of sludge took place in the test units. It is postulated that in the proposed system these factors could be largely controlled, except at the effluent end of the growth channel. At that point, provisions could be made for removing accumulating sludge.

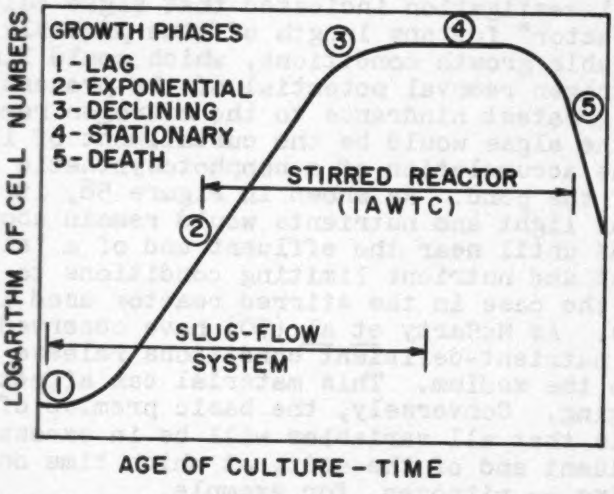

FIGURE 59 - THEORETICAL ALGAL GROWTH PHASES IN TWO TYPES OF ALGAL REACTORS

In Figure 60 is a diagrammatic sketch of a proposed algal nitrogen removal system which incorporates features that should result in the correction of many of the apparent inadequacies of the mixed system.

Basically, the influent tile drainage would enter a deep, one- to two-day detention time regulatory pond. This unit would provide: (1) buffering capacity, (2) a place for settling of silt and detritus and thus enhance light penetration, (3) a uniform flow to the treatment units, and (4) a means of isolating incoming flow when required. Algal "reseeding" probably would also be done after iron is added in this unit. Although the algal inoculum would consist mainly of actively growing cells (from the tailworks of the plant), some settling of older cells would also probably occur. A similar unit(s) would be provided to store flow during high nitrogen loading-low light periods of the year for more efficient treatment at a later time. Phosphorus could be injected into the overflow from the unit. The amount added at this point would, of course, depend upon influent nitrogen and alkalinity, and whether or not treatment was to be induced in the drain itself (15).

At this point, it might also be necessary to provide low-head pumping into the distribution canal. In any case, tile drainage would basically move by gravity flow through the treatment units at the required depth and detention time. (Head gates to individual units could be used selectively.) Although gates would probably be provided between each channel to allow series flow and reseeding after cleaning, the normal treatment pattern would be slug flow.

Data available at this time indicate that because deeper growth units are more efficient than shallower units of equal surface area, the use of deeper ponds would be the most economical approach in terms of land and construction costs. Possibly, depth could be graduated throughout the units, that is, deeper at the upper end and shallower near the outlet where algal concentration would be maximum.

It is further postulated that by treating the water in a slug-flow manner, auto-flocculation (due to high pH levels) and sludge buildup would also be minimal throughout most of the units. Most of the precipitation would occur toward the end of the channel, since amount of sedimentation is basically a function of algal culture age. A small settling area (approximately 100 x 100 x 8 feet) would be provided in each unit. Supernatant from this area would then overflow into a common tailwater canal which would carry the treated water to the separation area. The algal inoculum for the plant influent could be recycled from this point or from the separation unit itself.

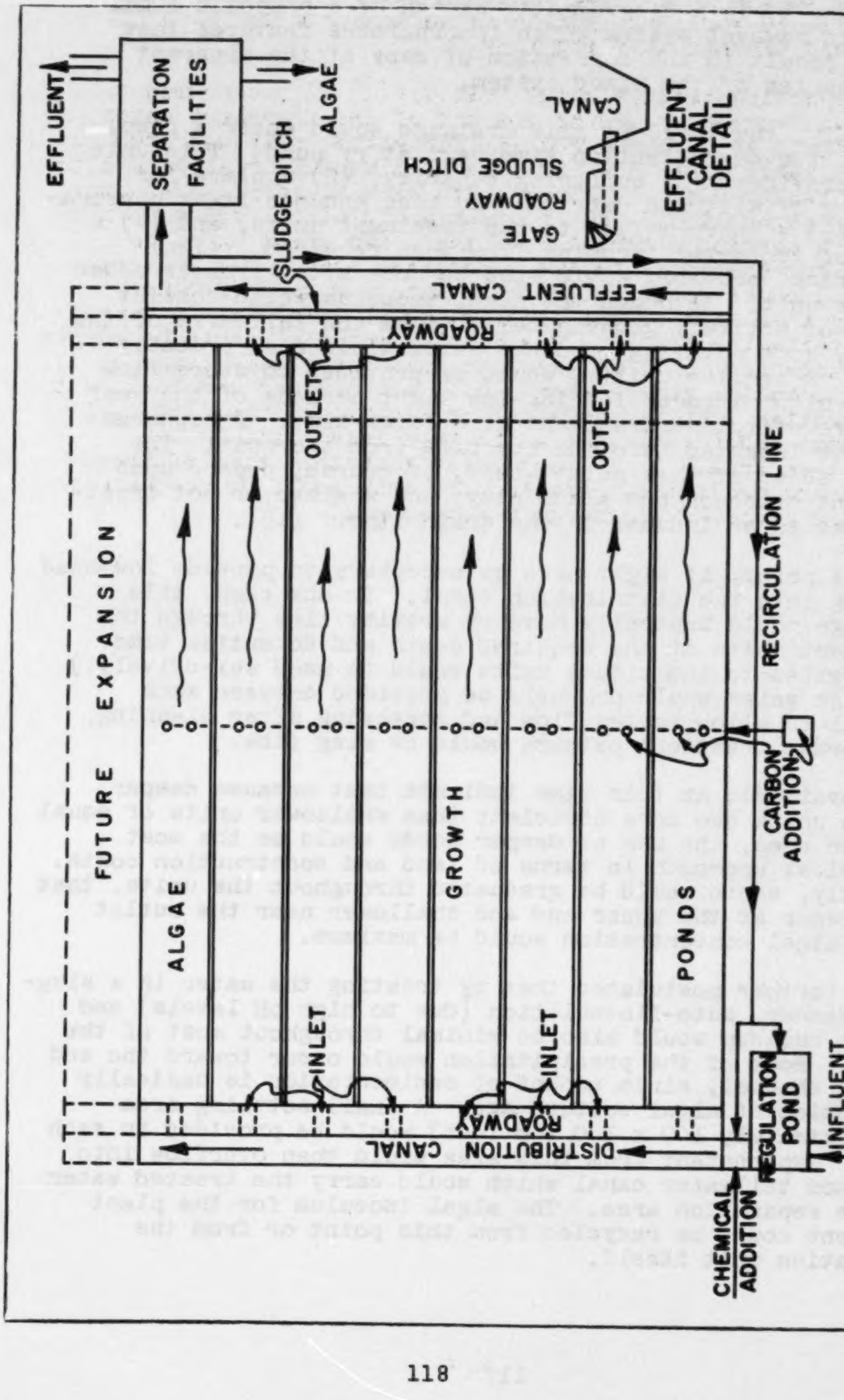
EFFLUENT
SEPARATION FACILITIES
ALGAE
SLUDGE DITCH
GATE
ROADWAY
SLUDGE DITCH
CANAL
EFFLUENT CANAL DETAIL
EFFLUENT CANAL
ROADWAY
OUTLET
OUTLET
FUTURE EXPANSION
ALGAE GROWTH PONDS
RECIRCULATION LINE
CARBON ADDITION
INLET
INLET
ROADWAY
DISTRIBUTION CANAL
REGULATION POND
INFLUENT
CHEMICAL ADDITION

FIGURE 60 - SCHEMATIC PLAN OF PROPOSED SLUG FLOW SYSTEM

At either end of the treatment channels would be an access roadway to the individual units to allow for maintenance. As mentioned earlier, individual units could be taken "off-line" and cleaned when required.

An interesting feature about this design is the common tailwater collection system at the end of the treatment units. There would be a common roadway for either railed or rubber-wheeled vehicles to the "in-line" sedimentation areas and to the tailwater ditch. (These units also would provide some primary separation.) The roadway would have a common canal in which settled material from these two areas could be pumped for transport to the separation area. The primary sludge product would undoubtedly have a much different quality than that of the algal material remaining in suspension (tailwater canal). The settled material could be pumped via a motorized unit on the road into the sludge canal on a periodic basis, and/or when a unit was drained and taken out of operation. Normal chemical or mechanical separation of the algae-laden tailwater would then take place in the separation area. It is postulated that this type of treatment using projected operating criteria would be adequate to meet the proposed discharge requirements.

ACKNOWLEDGMENTS

This phase of the field investigation concerned with nitrogen removal from tile drainage was performed under the joint direction of Messrs. Donald G. Swain, Sanitary Engineer, U. S. Bureau of Reclamation; Bryan R. Sword, Sanitary Engineer, Environmental Protection Agency; and Douglas L. Walker, Civil Engineer, California Department of Water Resources.

The field work was under the direction of Messrs. James F. Arthur, Research Aquatic Biologist, Environmental Protection Agency; and Bruce A. Butterfield, Assistant Engineer, California Department of Water Resources.

The cooperation and assistance given by the interagency staff of the treatment center was a major contribution to the success of the field studies. These personnel were:

Robert G. Seals	Chemist, Environmental Protection Agency
Norman W. Cederquist	Technician, U. S. Bureau of Reclamation
Gary E. Keller	Technician, U. S. Bureau of Reclamation
Dennis L. Salisbury	Technician, California Department of Water Resources
Elizabeth J. Boone	Laboratory Aid, California Department of Water Resources
Clara P. Hatcher	Laboratory Aid, California Department of Water Resources

Consultants to the Project

Dr. William J. Oswald	University of California
Dr. Clarence G. Golueke	University of California
Dr. Perry L. McCarty	Stanford University

Report Prepared by

James F. Arthur	Research Aquatic Biologist, Environmental Protection Agency

Report Edited by

Randall L. Brown	Associate Water Quality Biologist, California Department of Water Resources
Louis A. Beck	Senior Sanitary Engineer, California Department of Water Resources
G. Donald Meixner	Supervising Engineer, California Department of Water Resources

Preceding page blank

REFERENCES

1. California Department of Water Resources. "San Joaquin Master Drain". Bulletin No. 127. January 1965.

2. California Department of Water Resources. "Waste Water Quality, Treatment and Disposal". Bulletin No. 127, Appendix D. April 1969.

3. San Joaquin Valley Drainage Advisory Group, "Final Report", January 1969.

4. Department of Water Resources, Bulletin No. 174-2, "Progress Report, San Joaquin Valley Drainage Investigation Quality and Treatment Studies through December 31, 1967", August 1968.

5. Beck, Louis A., and Percy P. St. Amant, Jr. "Is Treatment of Agricultural Waste Water Possible?" Presented at Fourth International Water Quality Symposium, San Francisco, California; published in the proceedings of the meeting; August 14, 1968.

6. St. Amant, Jr., Percy P., and Louis A. Beck. "Nitrate Removal Studies at the Interagency Agricultural Waste Water Treatment Center, Firebaugh, California". Presented at 1969 Conference, California Water Pollution Control Association, Anaheim, California, and published in the proceedings of the meeting; May 9, 1969.

7. St. Amant, Jr., Percy P., and Louis A. Beck. "Research on Methods of Removing Excess Plant Nutrients from Water". Presented at 158th National Meeting and Chemical Exposition, American Chemical Society, New York, New York; September 8, 1969.

8. St. Amant, Jr., Percy P., and Perry L. McCarty. "Treatment of High Nitrate Waters". Presented at Annual Conference, American Water Works Association, San Diego, California; May 21, 1969. American Water Works Association Journal. Vol. 61. No. 12. pp. 659-662; December 1969.

9. "Effects of the San Joaquin Master Drain on Water Quality of the San Francisco Bay and Delta", Central Pacific Basins Comprehensive Water Pollution Control Project, U. S. Department of the Interior, Federal Water Pollution Control Administration, Southwest Region, January 1967.

Preceding page blank

10. "Effects of the San Joaquin Master Drain on Water Quality of the San Francisco Bay and Delta", Central Pacific Basins Comprehensive Water Pollution Control Project, Appendix Part C, "Nutrients and Biological Response", August 1968.

11. Brown, Randall L. "Removal of Nitrate by an Algal System", California Department of Water Resources Bulletin No. 174-10. June 1970.

12. Beck, Louis A., Percy P. St. Amant, Jr., and Thomas A. Tamblyn. "Comparison of Nitrate Removal Methods". Presented at Water Pollution Control Federation Meeting, Dallas, Texas. October 9, 1969.

13. Brown, Randall L., Richard C. Bain, Jr., and Milton G. Tunzi. "The Effects of Nitrogen Removal on the Algal Growth Potential of San Joaquin Valley Agricultural Tile Drainage Effluents" in Collected Papers Regarding Nitrates in Agricultural Waste Water, U. S. Government Printing Office, 1969.

14. Butterfield, Bruce A., and James R. Jones. "Harvesting of Algae Grown in Agricultural Wastewaters" in Collected Papers Regarding Nitrates in Agricultural Waste Water, U. S. Government Printing Office, 1969.

15. Goldman, Joel C., James F. Arthur, William J. Oswald, and Louis A. Beck. "Combined Nutrient Removal and Transport System for Tile Drainage from the San Joaquin Valley" in Collected Papers Regarding Nitrates in Agricultural Waste Water, U. S. Government Printing Office, 1969.

16. Arthur, James F., Randall L. Brown, Bruce A. Butterfield, and Joel C. Goldman. "Algal Nutrient Responses in Agricultural Wastewater" in Collected Papers Regarding Nitrates in Agricultural Waste Water, U. S. Government Printing Office, 1969.

17. Beck, Louis A., W. J. Oswald, and Joel C. Goldman. "Nitrate Removal from Agricultural Tile Drainage by Photosynthetic Systems". Presented at the American Society of Civil Engineers, Second National Symposium on Sanitary Engineering Research, Development and Design, July 15, 1969, Cornell University, Ithaca, New York.

18. Golueke, C. G., W. J. Oswald, and H. K. Gee. "Harvesting and Processing Sewage-grown Planktonic Algae". San. Eng. Res. Lab., University of California, Berkeley. SERL Report No. 64-8. May 1968.

19. Shelef, G., W. J. Oswald, and C. G. Golueke. "Kinetics of Algal Systems in Waste Treatment; Light Intensity and Nitrogen Concentration as Growth-limiting Factors", SERL Report No. 68-4, May 1968.

20. Jewell, William J., and Perry L. McCarty. "Aerobic Decomposition of Algae and Nutrient Regeneration". Department of Civil Engineering, Stanford University, Stanford, California, Technical Report No. 91, June 1968.

21. McCarty, Perry L. "Chemistry of Nitrogen and Phosphorus in Water", Journal of American Water Works Association, Vol. 62, No. 2. 1969.

22. Stumm, W., and J. O. Leckie. "Phosphate Exchange With Sediments; Its Role in the Productivity of Surface Waters". Presented at the Fifth International Water Pollution Research Conference. (In print) July-August 1970.

23. Cramer, Marian, and Jack Myers. "Nitrate Reduction and Assimilation in Chlorella". Journal of General Physiology, Vol. 32, 1949.

24. Bongers, L. H. J. "Kinetic Aspects of Nitrate Reduction", Netherlands Journal of Agricultural Science, Vol. 6, 1958.

25. Shelef, G., W. J. Oswald, and C. G. Golueke. "The Continuous Culture of Algal Biomass on Wastes". Presented at the Third International Symposium on Continuous Cultivation of Microorganisms; Prague, Czechoslovakia, June 17-23, 1968.

26. Golterman, H. L. (ed.). "Influence of the Mud on the Chemistry of Water in Relation to Productivity". Presented for the IBP Technical Meeting, "Chemical Environment in the Aquatic Habitat", October 10-16, 1966.

27. Detchum, Bostwich H. "Mineral Nutrition of Phytoplankton". Ann. Review of Plant Physiology, Vol. 5, 1954.

28. Lewin, Ralph A. (ed.). Physiology and Biochemistry of Algae, Academis Press, New York, 1962.

29. Foree, Edward G. "Algal Growth and Decomposition: Effects on Water Quality. Nutrient Uptake and Chemical Composition of Algae in Batch Culture". Research Report No. 27, University of Kentucky Water Resources Institute, Lexington, Kentucky, 1970.

30. Foree, Edward G., and Perry L. McCarty. "The Decomposition of Algae in Anaerobic Waters". Department of Civil Engineering, Stanford University, Stanford, California, Technical Report No. 95, August 1968.

31. Oswald, W. J., and C. G. Golueke. "Biological Transformation of Solar Energy", Adv. in Applied Microbiology, Vol. II, Academic Press, New York, 1960.

32. Oswald, William J. "Growth Characteristics of Microalgae Cultured in Domestic Sewage: Environmental Effects on Productivity". Presented before the IBP/PP Technical Meeting, "Productivity of Photosynthetic Systems, Part I, Models and Methods", Trebon, Czechoslovakia, September 19, 1969.

33. Krauss, R. W. "Physiology of the Fresh-water Algae", Ann. Rev. Agnt., Physiol., 9:207-244, 1958.

34. Kessler, Erick. "Nitrate Assimilation by Plants". Rev. Plant Physiology, Vol. 15, 1964.

35. Syrett, P. J. "The Assimilation of Ammonia and Nitrate by Nitrogen-starved Cells of Chlorella vulgaris; II. The Assimilation of Large Quantities of Nitrogen", Physiologia Plantarum, Vol. 9, 1956.

36. Fogg, G. E. Algal Cultures and Phytoplankton Ecology, University of Wisconsin Press, Madison and Milwaukee, 1965.

37. Borchardt, Jack A., and Hardam S. Azad. "Biological Extraction of Nutrients". Presented at the 40th Annual Conference of the Water Pollution Control Federation, New York City, New York, October 1967.

38. Tew, R. W., J. O. Sane, and R. P. Geckler. "Highly Concentrated Carbon Dioxide as a Carbon Source for Continuous Algae Cultures". Presented at Biologists for Space System Symposium, Technical Documentary Report No. AMRL-TDR-62-116; Biomedical Lab., 6570th Aerospace Medical Res. Lab., October 1962.

39. Pipes, Wesley O. "Carbon Dioxide-limited Growth of Chlorella in Continuous Culture". Presented at Winter Meeting of Society of Illinois Bacteriologists, Evanston, Illinois, February 17, 1962.

40. American Public Health Association. Standard Methods for the Examination of Water and Waste Water, 12th Edition, 1965.

41. Brown, Randall L., and James F. Arthur. "Effect of Surface/Volume Relationship, CO$_2$ Addition, Aeration, and Mixing on Nitrate Utilization by *Scenedesmus* Cultures in Subsurface Agricultural Waste Water". Proc. of the Eutrophication - Biostimulation Assessment Workshop, Middlebrooks, et al (ed.), 1969.

42. Provasoli, L., and I. J. Pinter. "Artificial Media for Fresh-water Algae: Problems and Suggestions" in *The Ecology of Algae*, Special Problems #2. *Pymatuning Laboratory of Field Biology*, University of Pittsburg, Pittsburg, Ohio. C. A. Pryon, Jr. and R. T. Hartman (ed.). April 1960.

43. Fruh, E. Gus. "The Overall Picture of Eutrophication". Presented at the Annual Short School of the Texas Water and Sewage Works Association, College Station, Texas, March 9, 1966.

44. Goldman, Charles R. (ed.). "Micronutrient Limiting Factors and Their Detection in Natural Phytoplankton Populations". Proceedings of IBP Symposium on Primary Productivity in Aquatic Environments; Pallanza, Italy, April 1965.

45. Hutner, S. H., L. Provasoli, Albert Schatz, and C. P. Haskins. "Some Approaches to the Study of the Role of Metals in the Metabolism of Microorganisms", *Proceedings of the American Philosophical Society*, *Vol. 94, No. 2, April 1950.*

46. Glandon, L. R. "Monitoring Nutrient and Pesticides in Subsurface Agricultural Drainage" in Collected Papers Regarding Nitrates in Agricultural Waste Water, U. S. Government Printing Office, 1969.

47. Gates, W. E., and J. A. Borchardt. "Nitrogen and Phosphorus Extraction from Domestic Wastewater Treatment Plant Effluents by Controlled Algal Culture", *Journal Water Pollution Control Federation*, April 1964.

48. McGauhey, P. H. "Engineering Management of Water Quality". Chapter 17. McGraw-Hill, Inc., New York. 1968.

49. Warburg, O., and E. Negelein. "The Transformation of Energy During the Assimilation of Carbon Dioxide". *Naturwissenschaften* 10:647-653. A. Physik Chem. *102:235-266. 1920.*

50. Davis, Edwin A. "Nitrate Reduction by *Chlorella*", *Plant Physiology*, Vol. 28, 1953.

51. Azad, Hardam S., and Jack A. Borchardt. "A Method for Predicting the Effects of Light Intensity on Algal Growth and Phosphorus Assimilation". Presented at the 41st Annual Conference of the Water Pollution Control Federation, September 22-27, 1968.

52. Emerson, Robert, and Charlton M. Lewis. "Factors Influencing the Efficiency of Photosynthesis", *American Journal of Botany*, Vol. 26, 1939.

53. Oswald, William J. "Light Conversion Efficiency of Algae Grown in Sewage". *Trans. Amer. Soc. Civil Engrs.* Vol. 128, Part III: 47-83. 1963.

54. Osterlind, S. "Inorganic carbon sources of green algae. II - Carbonic anhydrase in *Scenedesmus quadricauda* and *Chlorella pyrenoidosa*". *Physiologic Plantarum.* 3:430-434. 1950.

55. Gotaas, H. B., W. J. Oswald, and C. G. Golueke. "Algal-Bacterial Symbiosis in Sewage Oxidation Ponds". Fifth Program Report I.E.R. Ser. 44, Issue 5, Berkeley: Sanitary Eng. Research Lab., University of California, December 1954.

56. Hildebrand and Powell. "Carbonic Acid and Its Ions", *Principles of Chemistry*, MacMillan Co., Seventh Edition, 1964.

57. Ham, Robert K. "The Application of Some Fundamental Chemical Equilibrium Concepts to Water Systems", Water and Sewage Works, 1969.

58. *Handbook of Chemistry and Physics.* 41st Edition, Chemical Rubber Publishing Co., Cleveland, Ohio, 1959-60.

59. McKee, J. E., and H. W. Wolf. *Water Quality Criteria.* Publication 3A, the Resources Agency of California, State Water Quality Control Board, 1963.

60. Meyers, J. "Growth Characteristics of Algae in Relation to the Problems of Mass Culture", *Algal Culture from Laboratory to Pilot Plant*, J. S. Burlew (ed.), Publ. 600, Carnegie Inst. of Wash. pp. 204-232. 1953.

61. Bogan, R. H., O. E. Albertson, and J. C. Pluntze. "Use of Algae in Removing Phosphorus from Sewage", *Journal of the Sanitary Engineering Division, Proceedings of the American Society of Civil Engineers*, Vol. 86, No. SA 5, September 1960.

62. Levin, Gilbert V., and Joseph Shapiro. "Metabolic Uptake of Phosphorus by Wastewater Organisms", *Journal of WPCF*, Vol. 37, No. 6. 1965.

63. Eliassen, Rolf, and George Tchobanoglous. "Chemical Processing of Wastewater for Nutrient Removal", *Journal of Water Pollution Control Federation*, May 1968.

64. Lee, G. Fred. "Factors Affecting the Transfer of Materials Between Water and Sediments". University of Wisconsin, Water Resources Center, July 1970.

65. Zabat, M., W. Oswald, C. Golueke, and H. Gee. "Kinetics of Algae Systems in Waste Treatment, Phosphorus as a Growth Limiting Factor". SERL, University of California, Berkeley, California. September 1970.

66. Shapiro, Joseph. "Iron Available to Algae: Preliminary Report on a New Approach to its Estimation in Lake Water Through Use of the 'Ferrigram'". Presented and proc. of IBP Symposium, H. L. Golterman (ed.). October 10-16, 1966.

67. Hutchinson, H. E. "Redox [illegible]tential and the Iron Cycle", Chapter II, *A Treatise on L[illegible]ology*. John Wiley and Sons, New York, 1015 pp. 1967.

68. Morgan, J. J., and Werner Stumm. "The Role of Multivalent Metal Oxides in Limnological Transformations, as Exemplified by Iron and Manganese", Adv. in *Water Pollution Research*, Vol. I, 1964.

69. Delwiche, C. C. "The Nitrogen Cycle", *Scientific American*, September 1970.

70. Richardson, B., D. Orcutt, H. Schwertner, C. Martinez, and H. Wickline. "Effects of Nitrogen Limitation on the Growth and Composition of Unicellular Algae in Continuous Culture", *Applied Microbiology*, August 1969.

71. Fitzgerald, E. L., and Chin-Mien Ts'ai. "Algae Production and Waste Disposal in Taiwan" *Memoirs of the College of Medicine of National Taiwan University*, Vol. XV, No. 1, 1970.

72. Soong, Pinnan. "The Improvement of the Production and Utilization of Unicellular Algae in Taiwan", Annual Report Taiwan Sugar Experiment Station. 1969.

73. McGarry, M. "Application of the Sewage Grown Mass Algal Culture Process to the Asian Urban Environment", *The Treatment of Sewage and Production of Protein through the Mass Culture of Algae*, Research Program Report No. 6, Asian Institute of Technology, 1970.

PUBLICATIONS

SAN JOAQUIN PROJECT, FIREBAUGH, CALIFORNIA

1968

"Is Treatment of Agricultural Waste Water Possible?"
Louis A. Beck and Percy P. St. Amant, Jr. Presented at Fourth International Water Quality Symposium, San Francisco, California, August 14, 1968; published in the proceedings of the meeting.

1969

"Biological Denitrification of Wastewaters by Addition of Organic Materials"
Perry L. McCarty, Louis A. Beck, and Percy P. St. Amant, Jr. Presented at the 24th Annual Purdue Industrial Waste Conference, Purdue University, Lafayette, Indiana. May 6, 1969.

"Comparison of Nitrate Removal Methods"
Louis A. Beck, Percy P. St. Amant, Jr., and Thomas A. Tamblyn. Presented at Water Pollution Control Federation Meeting, Dallas, Texas. October 9, 1969.

"Effect of Surface/Volume Relationship, CO_2 Addition, Aeration, and Mixing on Nitrate Utilization by *Scenedesmus* Cultures in Subsurface Agricultural Waste Waters"
Randall L. Brown and James F. Arthur. Proceedings of the Eutrophication-Biostimulation Assessment Workshop, Berkeley, California. June 19-21, 1969.

"Nitrate Removal Studies at the Interagency Agricultural Waste Water Treatment Center, Firebaugh, California"
Percy P. St. Amant, Jr., and Louis A. Beck. Presented at 1969 Conference, California Water Pollution Control Association, Anaheim, California, and published in the proceedings of the meeting. May 9, 1969.

"Research on Methods of Removing Excess Plant Nutrients from Water"
Percy P. St. Amant, Jr., and Louis A. Beck. Presented at 158th National Meeting and Chemical Exposition, American Chemical Society, New York, New York. September 8, 1969.

"The Anaerobic Filter for the Denitrification of Agricultural Subsurface Drainage"
T. A. Tamblyn and B. R. Sword. Presented at the 24th Purdue Industrial Waste Conference, Lafayette, Indiana. May 5-8, 1969.

Preceding page blank

PUBLICATIONS
(Continued)

1969

"Nutrients in Agricultural Tile Drainage"
W. H. Pierce, L. A. Beck, and L. R. Glandon. Presented at the 1969 Winter Meeting of the American Society of Agricultural Engineers, Chicago, Illinois. December 9-12, 1969.

"Treatment of High Nitrate Waters"
Percy P. St. Amant, Jr., and Perry L. McCarty. Presented at Annual Conference, American Water Works Association, San Diego, California. May 21, 1969. American Water Works Association Journal. Vol. 61, No. 12. December 1969. pp. 659-662.

The following papers were presented at the National Fall Meeting of the American Geophysical Union, Hydrology Section, San Francisco, California. December 15-18, 1969. They are published in Collected Papers Regarding Nitrates in Agricultural Waste Water. USDI, FWQA, #13030 ELY, December 1969.

"The Effects of Nitrogen Removal on the Algal Growth Potential of San Joaquin Valley Agricultural Tile Drainage Effluents"
Randall L. Brown, Richard C. Bain, Jr., and Milton G. Tunzi.

"Harvesting of Algae Grown in Agricultural Wastewaters"
Bruce A. Butterfield and James R. Jones.

"Monitoring Nutrients and Pesticides in Subsurface Agricultural Drainage"
Lawrence R. Glandon, Jr., and Louis A. Beck.

"Combined Nutrient Removal and Transport System for Tile Drainage from the San Joaquin Valley"
Joel C. Goldman, James F. Arthur, William J. Oswald, and Louis A. Beck.

"Desalination of Irrigation Return Waters"
Bryan R. Sword.

"Bacterial Denitrification of Agricultural Tile Drainage"
Thomas A. Tamblyn, Perry L. McCarty, and Percy P. St. Amant, Jr.

"Algal Nutrient Responses in Agricultural Wastewater"
James F. Arthur, Randall L. Brown, Bruce A. Butterfield, and Joel C. Goldman.

1 *Accession Number*

W

2 *Subject Field & Group*

05 D

SELECTED WATER RESOURCES ABSTRACTS
INPUT TRANSACTION FORM

5 *Organization*

Department of Water Resources
San Joaquin District
Fresno, California

6 *Title*

REMOVAL OF NITRATE BY AN ALGAL SYSTEM

10 *Author(s)*

Arthur, James F.

16 *Project Designation*

13030 ELY 06/71-13

21 *Note*

Available from Department of Water Resources
Post Office Box 2385
Fresno, California 93723

22 *Citation*

Bio-engineering Aspects of Agricultural Drainage - San Joaquin Valley, California. Report No. 13030 ELY 06/71-13
Pages 132, Figures 60, Tables 16, References 73

23 *Descriptors (Starred First)*

*Agricultural Wastes, *Water Pollution Control, Biological Treatment, Nitrates, Treatment Facilities

25 *Identifiers (Starred First)*

*Algae Stripping, Scenedesmus, Algal Growth and Harvesting

27 *Abstract*

Major findings are presented from a one-year operational investigation conducted at the Interagency Agricultural Wastewater Treatment Center (IAWTC) on the use of algae to remove nitrogen from surface agricultural tile drainage in the San Joaquin Valley of California. The objectives of the study were to: (1) refine the design criteria, determined in a preliminary investigation, (2) develop operational procedures, and (3) recommend a design for a prototype algal nitrogen removal process.// The investigation demonstrated that the governing factors affecting the algal nitrogen removal process are the total amount of light available to the actively photosynthesizing algae and the influent nitrogen loading. Accordingly, if these two factors are known, the area required for nitrogen removal can be approximated.// Turbid conditions, resulting from the suspension of nonphotosynthesizing material during continuous or intermittent mixing, were found to be detrimental to the prolonged operation of the system. Maximum nitrogen assimilation also depended upon providing a completely balanced nutrient system, and varying amounts of supplemental carbon, phosphorus, and iron were required throughout the year.// Algae harvesting studies indicated that 90 percent or more of the algae could be removed throughout the year, under continuous operation, using a chemical-flocculent-sedimentation process but that the chemical additions required were dependent upon a number of algal growth factors.// Continuous operation of algal test units during 1970 showed the algal nitrogen removal process was capable of effectively reducing the influent nitrate-nitrogen concentration as well as other plant nutrients. The process reduced a varying influent nitrogen concentration of from 15 to 30 mg/l NO_3-N to 2 to 4 mg/l soluble effluent nitrogen throughout the year using varying operating parameters.// Recommendations are also given for the design and testing of "prototype" algal nitrogen removal plants using a modification of the stirred reactor design, and a proposed "slug-flow" algal nitrogen removal system designed to correct many of the inadequacies inherent in the first system.

Abstractor Arthur

Institution Department of Water Resources

WR:102 (REV. JULY 1969)
WRSIC

SEND, WITH COPY OF DOCUMENT, TO: WATER RESOURCES SCIENTIFIC INFORMATION CENTER
U.S. DEPARTMENT OF THE INTERIOR
WASHINGTON, D. C. 20240

*U.S. GOVERNMENT PRINTING OFFICE: 1973 514-152/176 1-3